Effect of KCl substitution on bacterial viability of *Escherichia coli* (ATCC 25922) and selected probiotics

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ABSTRACT

Excessive intake of NaCl has been associated with the increased risk of several diseases, particularly hypertension. Strategies to reduce sodium intake include substitution of NaCl with other salts, such as KCl. In this study, the effects of NaCl reduction and its substitution with KCl on cell membranes of a cheese starter bacterium (*Lactococcus lactis* ssp. *lactis*), probiotic bacteria (*Bifidobacterium longum*, *Lactobacillus acidophilus*, and *Lactobacillus casei*), and a pathogenic bacterium (*Escherichia coli*) were investigated using Fourier-transform infrared (FTIR) spectroscopy. A critical NaCl concentration that inhibited the viability of *E. coli* without affecting the viability of probiotic bacteria significantly was determined. To find the critical NaCl concentration, de Man, Rogosa, and Sharpe (MRS) broth was supplemented with a range of NaCl concentrations [0 (control), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0%], and the effect on cell viability and FTIR spectra was monitored for all bacteria. A NaCl concentration of 2.5% was found to be the critical level of NaCl to inhibit *E. coli* without significantly affecting the viability of most of the probiotic bacteria and the cheese starter bacterium. The FTIR spectral analysis also highlighted the changes that occurred mainly in the amide regions upon increasing the NaCl concentration from 2.5 to 3.0% in most of the bacteria. *Escherichia coli* and *B. longum* were more sensitive to substitution of NaCl with KCl, compared with *Lb. acidophilus*, *Lb. casei*, and *Lc. lactis* ssp. *lactis*. To evaluate the effect of substitution of NaCl with KCl, substitution was carried out at the critical total salt concentration (2.5%, wt/vol) at varying concentrations (0, 25, 50, 75, and 100% KCl). The findings suggest that 50% substitution of NaCl with KCl, at 2.5% total salt, could inhibit *E. coli* without affecting the probiotic bacteria.

Key words: NaCl reduction, KCl substitution, Fourier-transform infrared spectroscopy

INTRODUCTION

Table salt (NaCl) is the most common food additive that contributes not only to the texture and sensory profile, but also to the functional properties of food. Increased shelf life and improved flavor and texture are among the major contributions of salt addition in dairy products (Albarracin et al., 2011). In recent times, numerous attempts have been made to reduce the intake of salt because of the risks associated with excessive salt intake (Buemi et al., 2002; Kotchen, 2005; Massey, 2005; Heaney, 2006). One of the various strategies to reduce NaCl intake is its substitution by other salts, such as KCl, MgCl₂, and CaCl₂ (Fitzgerald and Buckley, 1985). However, because of the different effects of each cation, not all salt substitutions yield the desired characteristics in any product (Fitzgerald and Buckley, 1985), thus making it important to find the critical sodium reduction and substitution level. Among the dairy products, cheeses contain varying levels of salt and, thus, interest has increased in studying the effect of sodium reduction and its replacement on the taste and texture of various cheeses (Ayyash and Shah, 2011a,b). However, limited studies have examined the effects of NaCl on the structural changes in dairy bacteria, which may be important to understand their behavior and activity when exposed to salt.

Dairy products such as Labneh and cheeses contain varying levels of salt, which also affects the cell membrane of the bacteria, thereby affecting viability and activity. The effect of salt reduction and substitution on cell viability of selected bacteria has been evaluated (Nielsen and Zeuthen, 1987; Roy, 1991). Studies have shown that whereas divalent ions such as calcium affect the mitochondrial membranes, monovalent cations such as sodium and potassium have a significant effect on the properties of the plasma membrane (Altenbach and Seelig, 1984; Herbet et al., 1984). The effect of sodium ions on membrane phospholipids is reported to be stronger than potassium (Binder and Zschörnig, 2002; Fukuma et al., 2007; Gurtovenko and Vattulainen, 2008). Furthermore, KCl is the most common substitute, as it helps maintain salty taste while effectively reducing the amount of salt in food up to 25% (Cruz

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et al., 2011). It is thus important to examine the affect and extent of sodium reduction and substitution on the structural changes in bacteria and their survival to better understand their stress responses. Fourier-transform infrared (FTIR) spectroscopy has been used to study the changes occurring in biological macromolecules in response to the environmental stress (Santivarangkna et al., 2007). Fourier-transform infrared spectroscopy has proven to be a useful technique, particularly to study bacterial stress responses and microbial metabolism, as the microorganisms can be studied in their intact state (Naumann et al., 1991; Zeroual et al., 1994; Winson et al., 1997; Alvarez-Ordóñez et al., 2010).

The objectives of this study were to investigate the effects of NaCl reduction on the cell viability and structural changes of a starter bacterium (*Lactococcus lactis* ssp. *lactis*), 3 probiotic bacteria (*Bifidobacterium longum*, *Lactobacillus acidophilus*, and *Lactobacillus casei*), and *Escherichia coli*, and find a critical salt concentration. Critical salt concentration for the purpose of this study is defined as the salt concentration that would inhibit the viability of the *E. coli*, without significantly affecting the viability of the probiotic bacteria. Furthermore, the aim was to examine the effect of NaCl substitution with KCl at the critical total salt concentration, as stated above, and find an optimum substitution level, at which the viability of probiotic bacteria is maintained without increase in the viability of *E. coli*.

MATERIALS AND METHODS

Bacterial Cultivation

Lactobacillus acidophilus (CSCC 2400), *Lactobacillus casei* (ASCC 290), and *Bifidobacterium longum* (CSCC 5089) were obtained from the Australian Starter Culture Collection (Dairy Innovation Australia Ltd., Werribee, VIC, Australia) and were stored at -80°C . *Lactococcus lactis* ssp. *lactis* (Ward's 85W 1774) was obtained from the Chinese University of Hong Kong (Shatin, Hong Kong). *Escherichia coli* (ATCC 25922) was provided by the University of Hong Kong (Pokfulam, Hong Kong). *Escherichia coli* was activated in Luria-Bertani broth, *Lc. lactis* ssp. *lactis* in M17 broth, and the remaining organisms were activated in sterile de Man, Rogosa, and Sharpe (MRS) broth (Becton Dickinson and Co., Franklin Lakes, NJ) by 1% inoculation and then incubation at 37°C for 24 h. The activated organisms were used after 3 successive transfers in sterile MRS broth.

Experimental Design

To find a critical NaCl concentration that inhibits *E. coli* without affecting the probiotic organisms, a range

of NaCl concentrations was selected. Sterile MRS broth with varying NaCl concentrations [0% (control), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0% (wt/vol)] was inoculated with 1% (vol/vol) activated culture and incubated at 37°C for 24 h. An aliquot (200 μL) of the medium was used to estimate the number of colony-forming units, and the remainder was used for FTIR spectroscopic analysis.

To evaluate the effect of substitution of NaCl with KCl, substitution was carried out at the critical total salt concentration, as determined by the abovementioned method. Varying substitution concentrations [0, 25, 50, 75, and 100% KCl (wt/wt)] were used in this study.

Determination of Cell Viability

After 24 h, the cell viability of each sample was determined. For enumeration of the viable cells, serial dilutions of each sample were made in sterile peptone water (0.15%, wt/vol) and were spread on MRS agar plates for *Lb. acidophilus* and *Lb. casei*, M17 agar plates for *Lc. lactis* ssp. *lactis*, MRS-cysteine agar plates for *B. longum*, and Luria-Bertani agar plates for *E. coli* (Goodridge et al., 1999). The plates were incubated for 48 h at 37°C (in an anaerobic jar for *B. longum*) and the colony-forming units were enumerated.

FTIR Spectroscopic Study

FTIR Analysis. Sample preparation was carried out according to Beattie et al. (1998). Briefly, the culture medium was centrifuged at $4,500 \times g$ for 30 min at 4°C and the bacterial cell pellet was washed twice by resuspension in sterile physiological saline (0.90%) and recovered by centrifugation ($4,000 \times g$ for 10 min at 4°C). The washed cells were suspended in sterile distilled water to a concentration of 100 mg wet cells/mL. Fifty microliters of this fresh bacterial suspension was placed on a CaF_2 optical plate and was dried in an oven at 50°C for 30 min.

All spectra were recorded using an FTIR spectrophotometer [deuterated L-alanine doped triglycine sulfate (DLATGs) detector, mid-infrared (MIR) source, and OPUS 6.5 software; Bruker Corp., Billerica, MA] at a resolution of 4 cm^{-1} and an average of 20 scans from 4,000 to $1,000\text{ cm}^{-1}$ at room temperature (20°C) in a controlled environment (relative humidity of 40 to 50%). The spectra were transformed (baseline corrected, smoothed, and normalized using the Savitzky-Golay algorithm) and peaks were detected (curve-fitting algorithm) above the threshold of 0.50%. To correct for background, a spectrum of the air was recorded before each sample.

FTIR Spectral Band Assignment. Fourier-transform infrared spectroscopic analysis revealed information about the changes in functional groups at the surface of the bacterial membrane. The tentative assignments of bands by Naumann et al. (1991) and Cai and Singh (1999) were considered for assignment of major bands. The region between 4,000 to 3,100 cm^{-1} is representative of broad conformational features, with bands at around 3,300 cm^{-1} ascribed to N–H stretching modes of the amide-A group. A linear relationship exists between the N–H stretching frequency in the 3,200- to 3,300- cm^{-1} region and the distance from the nitrogen to the oxygen atom was suggested by Krimm and Bandekar (1986). The regions between 3,000 and 2,800 cm^{-1} are assigned to the alkyl hydrocarbons and bands for the FA region are expected at around 2,960, 2,924, and 2,853 cm^{-1} and these represent asymmetric CH_3 stretching, asymmetric CH_2 stretching, and symmetric CH_2 stretching in the membrane FA (Naumann et al., 1991).

Amide-I and amide-II regions, at 1,700 to 1,600 cm^{-1} and approximately 1,550 cm^{-1} respectively, represent carbonyl stretching of secondary amides and N–H bending, respectively; both these regions are representative of cell wall proteins. In the amide-I region, the peak at approximately 1,658 cm^{-1} is associated with random coils, 1,652 to 1,648 cm^{-1} is assigned to α -helices, and 1,648 to 1,642 cm^{-1} is assigned to irregular structures (Hussain et al., 2011). The intensity of the amide-I band is contributed by the C=O stretching vibrations (70–85%) and C–N stretching vibrations (10–20%; Kilimann et al., 2006). A decrease in the frequency of amide I is associated with a decrease in the strength of hydrogen bonding of the C=O acceptor (Takeda et al., 1995a,b). Furthermore, an amide-I band at approximately 1,636 cm^{-1} can be representative of β -sheets. The amide-II region appears around 1,550 cm^{-1} and the band at 1,453 cm^{-1} , also referred as amide II' (Kilimann et al., 2006), is attributed to the greater CH_2 deformation in proteins.

Tentative assignments of the amide-III region (1,330–1,220 cm^{-1}), as carried out by Cai and Singh (1999), suggest that the region from 1,250 to 1,220 cm^{-1} is attributed to β -sheets. Conventionally, the amide-I region was the most widely used for determination of protein structure, because of its strong signal. However, later it was suggested that the amide-III region is more sensitive to the secondary structure of proteins and provides better prediction for the structure of the proteins compared with the amide-I region (Cai and Singh, 2004; Hussain et al., 2011).

Statistical Analysis

All experiments were replicated 3 times and all analyses were carried out in duplicate. The data obtained

were analyzed using one-way ANOVA at the 95% level of significance, using SPSS statistics software (version 20.0; IBM Corp., Armonk, NY). The Tukey post-hoc analysis was performed to further investigate the difference between the means at different NaCl concentrations and KCl substitution levels (Vermeirssen et al., 2003).

RESULTS AND DISCUSSION

Cell Viability

Effect of NaCl Reduction: Determination of a Critical Salt Concentration. The effect of NaCl reduction on the viable cell count ($\log_{10}$ cfu/mL) of bacteria is shown in Figure 1. An increase in NaCl concentration was inversely associated with the cell viability for all the bacteria, similar to the results of Beresford et al. (2001). In *E. coli*, a significant ($P < 0.05$) decrease in cell viability was observed upon increasing the concentration from 2.0 to 2.5%, and on further increase to 3.0% NaCl, no significant difference was observed compared with that containing 2.5% NaCl. However, *B. longum* showed no significant ($P > 0.05$) decrease upon increasing the salt content to 2.5%, but its viability decreased significantly at 3.0% NaCl. A similar trend was observed in other studies where cell viability was inversely affected by high NaCl concentrations in *Lactobacillus rhamnosus* (Prasad et al., 2003) and *Lactobacillus helveticus* (Roy, 1991). This study indicated that at 2.5% NaCl, the viability of *E. coli* was significantly ($P < 0.05$) reduced compared with 2.0% NaCl, with no significant difference in *B. longum*, *Lc. lactis* ssp. *lactis*, and *Lb. acidophilus*. Although a significant ($P < 0.05$) decrease in *Lb. casei* was also observed upon increasing NaCl from 2.0 to 2.5%, 2.5% was still considered as the critical concentration, as it was able to maintain the viability of most of the organisms used in this study and inhibited *E. coli*.

Effect of NaCl Substitution with KCl. The critical concentration of NaCl (2.5%) was taken as the salt concentration for its substitution with KCl. The cell viability as affected by substitution of NaCl with KCl is expressed as the logarithm of colony-forming units per milliliter in Figure 2. The viability of *Lc. lactis* ssp. *lactis* and *Lb. acidophilus* was not significantly ($P > 0.05$) affected by KCl substitution. This could be indicative of their ability to grow in modified salt in the media. In general, as the KCl levels increased, an increase in the cell viability occurred. *Lactobacillus casei* showed significantly ($P < 0.05$) increased cell count at 75 and 100% KCl compared with the control group (0% KCl). However, *E. coli* and *B. longum* were found to be more sensitive to substitution by KCl compared with

Figure 1. Effect of NaCl reduction on viable cell count ($\log_{10}$ cfu/mL). Values are means $\pm$ SE of at least 4 replicates ($n \geq 4$). Initial cell count ($\log_{10}$ cfu/mL): *Escherichia coli* (EC): 8.42 ± 0.08 ; *Bifidobacterium longum* (BL): 9.21 ± 0.07 ; *Lactobacillus acidophilus* (LA): 9.67 ± 0.12 ; *Lactobacillus casei* (LC): 10.17 ± 0.05 ; *Lactococcus lactis* ssp. *lactis* (SL): 9.64 ± 0.31 . Values for each bacterium with the same letter (a–e) do not differ significantly ($P > 0.05$). Color version available in the online PDF.

the other bacteria. A significant decrease was observed on reducing the KCl substitution from 75 to 50%. In *E. coli*, a significant increase was observed in 25% ($7.95 \log_{10}$ cfu/mL) and 50% ($7.99 \log_{10}$ cfu/mL) KCl compared with the control ($7.76 \log_{10}$ cfu/mL; 0% KCl) and a further increase to $8.26 \log_{10}$ cfu/mL in 100% KCl was shown. *Bifidobacterium longum* also showed a significant increase in cell count with the increased KCl concentration, with the highest cell viability at 100% KCl ($9.65 \log_{10}$ cfu/mL). Nielsen and Zeuthen (1987) showed that substitution of NaCl with other salts such as CaCl_2 and MgCl_2 has the potential to inhibit pathogenic and saprophytic bacteria. Their study concluded that the inhibitory effect of substitution of NaCl by KCl or CaCl_2 could be similar to NaCl and is also dependent on bacteria. Further, the study by Roy (1991) revealed that NaCl and CaCl_2 inhibited the growth of *Lactobacillus helveticus* strain milano more than KCl. Substitution of NaCl by KCl at 50% in the current study was considered optimum, as the number of *E. coli* increased significantly on further increasing the KCl concentration, and no significant increase in the probiotic bacteria was observed in substitution beyond 50%.

FTIR Spectroscopy

Effect of NaCl Reduction on FTIR Spectra. Fourier-transform infrared spectra for each of the

samples was recorded, and the wavenumbers at which distinctive peaks (threshold of 0.50%) were observed are presented in Tables 1 to 5. For *E. coli*, distinctive spectra could not be recorded at higher NaCl concentrations (4.0, 4.5, and 5.0%) due to extremely reduced growth of the cells. A representative spectrum is shown in Figure 3 for *Lb. acidophilus* at 3 different NaCl concentrations (0, 2.5, and 5.0%).

The spectral changes as observed at varying NaCl concentrations for *Lc. lactis* ssp. *lactis* (Table 1) indicate overall stability of the amide A and the FA region on reducing the salt content, with no significant ($P > 0.05$) shifts in the wavenumbers. A significant increase in the peak frequencies in the amide-I region was observed upon increasing the NaCl concentration from 2.5 ($1,638 \text{ cm}^{-1}$) to 3.0% ($1,642 \text{ cm}^{-1}$). As per the tentative assignment by Kilimann et al. (2006), these wavenumbers are reflective of changes from β -sheets ($\sim 1,638 \text{ cm}^{-1}$) to irregular structures ($\sim 1,642 \text{ cm}^{-1}$). The changes in the amide-I region imply that 2.5% could be the critical NaCl concentration, as an increase in concentration to 3.0% may lead to changes in the protein structures. However, no significant changes were observed in amide-II and amide-II' regions of *Lc. lactis* ssp. *lactis*. The band associated with the β -sheets around $1,250$ to $1,220 \text{ cm}^{-1}$ in the amide-III region showed a gradual decrease in the frequency with the increase in the salt concentration.

Figure 2. Effect of NaCl substitution with KCl on viable cell count ($\log_{10}$ cfu/mL). Values are means $\pm$ SE of at least 4 replicates ($n \geq 4$). Initial cell count ($\log_{10}$ cfu/mL): *Escherichia coli* (EC): 8.42 ± 0.08 ; *Bifidobacterium longum* (BL): 9.21 ± 0.07 ; *Lactobacillus acidophilus* (LA): 9.67 ± 0.12 ; *Lactobacillus casei* (LC): 10.17 ± 0.05 ; *Lactococcus lactis* ssp. *lactis* (SL): 9.24 ± 0.31 . Values for each bacterium with the same letter (a–c) do not differ significantly ($P > 0.05$). Color version available in the online PDF.

The FTIR spectra as affected by salt reduction in *B. longum* are shown in Table 2. The amide-A region, with a peak at $3,286 \text{ cm}^{-1}$ remained unaffected upon increasing the NaCl to 2.5%, beyond which a significant increase to $3,289 \text{ cm}^{-1}$ was observed at 5.0% NaCl. This shift in the amide-A region is associated with N–H stretching modes of the peptide backbone (Krimm and Bandekar, 1986). The peaks associated with stretching of the FA region at $2,962$ and $2,924 \text{ cm}^{-1}$ showed stability upon increasing the NaCl concentration. However, the peak at $2,962 \text{ cm}^{-1}$ was not observed at 4.5 and 5.0% NaCl. This peak is attributed to asymmetric CH_3 stretching and is associated with the membrane FA. The CH_2 stretching at $2,853 \text{ cm}^{-1}$, which is associated with membrane fluidity, showed an insignificant decrease in the peak frequency and was not distinctively observed at 4.5 and 5.0% NaCl. This could imply that the higher salt content had affected the membrane lipid structure and membrane fluidity of the microorganism (Beney and Gervais, 2001). The amide regions of *B. longum* were sensitive to increasing salt concentrations. A sudden decrease in the wavenumber from $1,658$ to $1,652 \text{ cm}^{-1}$ was observed upon increasing the NaCl concentration beyond 2.5%. This is indicative of the possible changes in the random coils ($\sim 1,658 \text{ cm}^{-1}$) moving toward α -helices ($\sim 1,652$ – $1,648 \text{ cm}^{-1}$).

Table 3 shows the wavenumbers at which distinct peaks were observed in *Lb. acidophilus*. A significant increase in the frequency of the amide-A region was observed upon increasing the concentration from 2.5% ($3,284 \text{ cm}^{-1}$) to 3.0% NaCl ($3,287 \text{ cm}^{-1}$), indicative of stretching in the peptide backbone. The peak at $2,853 \text{ cm}^{-1}$, which is associated with membrane fluidity, was not affected by the increase in NaCl concentration up to 4.5% NaCl; however, this peak was absent in 5.0% NaCl. The amide-II and amide-III regions showed stability when *Lb. acidophilus* was subjected to higher NaCl concentrations.

It is interesting to note that *Lb. casei* was stable to changes in NaCl concentration (Table 4). Most of the functional groups showed no significant shift in the wavenumbers at which distinctive peaks appear for amide regions and the FA regions. A gradual decrease in the wavenumber was observed for the amide-III region of *Lb. casei* from $1,242 \text{ cm}^{-1}$ in the control to $1,240 \text{ cm}^{-1}$ in 5.0% NaCl, which is associated with the β -sheets structure.

The changes in FTIR spectra of *E. coli* as affected by varying salt concentrations are shown in Table 5. The amide-A region of *E. coli* showed a significant decrease in the wavenumbers upon increasing the NaCl concentration from 2.0 to 2.5%. The membrane fluidity ($2,853$

Figure 3. Fourier-transform infrared spectra for *Lactobacillus acidophilus* subjected to varying NaCl concentrations [0% (brown; middle spectrum in panel A; upper curve in panel B), 2.5% (orange; bottom spectrum in panel A; lightest curve in panel B), and 5.0% (blue, top spectrum in panel A)]. (A) The whole spectral range 4,000 to 1,000 cm^{-1} and (B) the region between 2,000 and 1,000 cm^{-1} . Color version available in the online PDF.

cm^{-1}) of *E. coli* was not affected upon increasing the salt concentration to 3.5% NaCl, as indicated by the band at 2,853 cm^{-1} . These findings are similar to those

reported by Alvarez-Ordóñez et al. (2010), who found no effect of varying NaCl concentration on the band at approximately 2,850 cm^{-1} in *Salmonella enterica* 'Ty-

Table 1. Wavenumbers (cm^{-1}) of distinct peaks of *Lactococcus lactis* ssp. *lactis* as affected by varying NaCl concentrations¹

NaCl (% wt/vol)										
0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0
3,285.94 ± 0.88	3,285.71 ± 0.61	3,286.29 ± 0.13	3,285.95 ± 0.17	3,285.87 ± 0.51	3,286.16 ± 0.50	3,284.36 ± 0.97	3,285.23 ± 0.47	3,289.67 ± 4.33	3,285.71 ± 0.85	3,282.32 ± 1.62
2,962.44 ± 0.19	2,961.70 ± 0.06	2,962.29 ± 0.21	2,961.72 ± 0.10	2,962.35 ± 0.08	2,962.14 ± 0.06	2,962.30 ± 0.08	2,962.33 ± 0.05	2,962.19 ± 0.08	2,962.26 ± 0.02	2,962.23 ± 0.12
2,925.13 ± 0.33	2,923.89 ± 0.11	2,924.47 ± 0.33	2,923.74 ± 0.08	2,924.40 ± 0.19	2,924.72 ± 0.13	2,924.65 ± 0.04	2,924.62 ± 0.02	2,924.59 ± 0.20	2,924.82 ± 0.21	2,924.78 ± 0.13
2,853.17 ± 0.06	2,852.90 ± 0.07	2,853.14 ± 0.05	2,852.91 ± 0.04	2,853.12 ± 0.04	2,853.18 ± 0.02	2,853.15 ± 0.03	2,853.09 ± 0.02	2,853.11 ± 0.02	2,853.11 ± 0.01	2,853.11 ± 0.02
1,662.71 ± 1.28	1,664.00 ± 2.01	1,662.05 ± 2.03	1,665.65 ± 0.02	1,657.64 ± 2.24	1,656.66 ± 1.90	1,659.33 ± 1.81	1,655.65 ± 1.44	1,662.63 ± 2.47	1,653.26 ± 2.57	1,656.31 ± 1.55
1,645.78 ± 2.38	1,649.16 ± 1.28	1,644.37 ± 2.04	1,647.54 ± 0.25	1,639.40 ± 2.02	1,638.55 ± 0.75	1,642.59 ± 2.71	1,645.46 ± 1.71	1,650.90 ± 3.42		
1,634.22 ± 1.29	1,632.67 ± 0.69	1,631.18 ± 1.05	1,631.40 ± 0.03	1,630.12 ± 0.57	1,631.94 ± 0.45	1,632.99 ± 1.59	1,634.91 ± 1.68	1,634.50 ± 2.15	1,635.98 ± 1.96	1,637.53 ± 1.80
1,551.99 ± 1.26	1,553.77 ± 0.30	1,551.24 ± 0.98	1,553.64 ± 0.10	1,548.23 ± 2.17	1,552.50 ± 1.05	1,551.10 ± 0.76	1,548.56 ± 2.72	1,551.36 ± 1.19	1,551.35 ± 1.92	
1,451.55 ± 2.49	1,454.54 ± 0.07	1,451.37 ± 2.01	1,454.10 ± 0.08	1,451.97 ± 2.09	1,453.85 ± 1.11	1,452.02 ± 1.71	1,453.59 ± 0.56	1,452.14 ± 1.66	1,453.91 ± 0.58	1,452.53 ± 1.69
1,244.13 ± 0.98	1,242.89 ± 0.18	1,243.21 ± 0.47	1,242.77 ± 0.15	1,242.55 ± 0.28	1,242.01 ± 0.13	1,241.70 ± 0.37	1,241.88 ± 0.04	1,241.19 ± 0.10	1,241.65 ± 0.15	1,240.71 ± 0.30
1,121.71 ± 0.72	1,120.30 ± 0.23	1,122.04 ± 0.47	1,120.43 ± 0.35	1,122.16 ± 0.28	1,122.25 ± 0.23	1,122.20 ± 0.14	1,122.21 ± 0.22	1,122.24 ± 0.11	1,122.34 ± 0.18	1,122.12 ± 0.19
1,084.49 ± 0.02	1,083.80 ± 0.12	1,084.64 ± 0.60	1,083.66 ± 0.12	1,085.00 ± 0.42	1,084.42 ± 0.51	1,084.80 ± 0.22	1,084.90 ± 0.28	1,084.43 ± 0.56	1,084.47 ± 0.56	1,084.83 ± 0.26

¹Values are means ± SE of 3 replicates.**Table 2.** Wavenumbers (cm^{-1}) of distinct peaks of *Bifidobacterium longum* as affected by varying NaCl concentrations¹

NaCl (% wt/vol)										
0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0
3,286.30 ± 0.08	3,285.06 ± 0.67	3,285.23 ± 0.11	3,285.01 ± 0.29	3,285.10 ± 0.06	3,285.47 ± 0.09	3,287.99 ± 1.55	3,287.25 ± 1.27	3,280.16 ± 1.45	3,291.64 ± 0.09	3,289.35 ± 2.54
2,961.58 ± 0.07	2,961.38 ± 0.04	2,961.38 ± 0.05	2,961.47 ± 0.04	2,961.37 ± 0.06	2,961.40 ± 0.07	2,960.19 ± 0.42	2,960.94 ± 0.30	2,961.11 ± 0.30		
2,924.29 ± 0.05	2,924.40 ± 0.06	2,924.36 ± 0.02	2,923.66 ± 0.20	2,924.17 ± 0.03	2,923.67 ± 0.19	2,921.92 ± 0.65	2,923.03 ± 0.23	2,923.96 ± 0.25	2,925.62 ± 0.35	2,923.63 ± 1.05
2,853.06 ± 0.02	2,853.10 ± 0.02	2,853.03 ± 0.02	2,852.79 ± 0.05	2,852.93 ± 0.01	2,852.72 ± 0.08	2,851.27 ± 0.38	2,852.00 ± 0.17	2,852.21 ± 0.13		
1,665.54 ± 0.13	1,665.83 ± 0.46	1,659.70 ± 0.39	1,660.93 ± 1.37	1,658.10 ± 0.11	1,658.22 ± 0.19					
1,646.24 ± 0.14	1,655.86 ± 0.50	1,653.30 ± 0.15	1,649.62 ± 1.79			1,652.71 ± 0.38	1,652.42 ± 0.55	1,652.50 ± 0.09	1,651.57 ± 0.20	1,652.24 ± 0.47
1,632.30 ± 0.22	1,636.76 ± 1.70	1,638.02 ± 0.65	1,635.21 ± 1.09	1,638.62 ± 0.41	1,638.51 ± 0.31	1,637.23 ± 0.13	1,636.97 ± 0.70	1,637.18 ± 0.15	1,636.49 ± 0.15	1,636.87 ± 0.27
1,552.68 ± 0.16	1,552.07 ± 0.32	1,550.57 ± 0.28	1,552.04 ± 0.79	1,549.70 ± 0.27	1,550.95 ± 0.28	1,554.63 ± 0.87	1,554.22 ± 0.01	1,555.64 ± 0.58	1,557.08 ± 0.24	1,554.17 ± 3.15
1,453.72 ± 0.06	1,455.20 ± 0.06	1,453.78 ± 0.03	1,454.45 ± 0.11	1,454.05 ± 0.07	1,453.87 ± 0.06	1,455.70 ± 0.30	1,455.84 ± 0.17	1,456.19 ± 0.15	1,456.63 ± 0.12	1,455.78 ± 0.55
1,242.62 ± 0.04	1,242.78 ± 0.07	1,242.56 ± 0.05	1,242.65 ± 0.12	1,242.76 ± 0.06	1,243.34 ± 0.06	1,242.54 ± 0.05	1,241.17 ± 0.15	1,241.66 ± 0.09	1,232.30 ± 0.10	1,237.15 ± 2.84
1,120.69 ± 0.18	1,120.83 ± 0.19	1,120.28 ± 0.09	1,121.67 ± 0.05	1,120.37 ± 0.26	1,120.26 ± 0.51	1,105.47 ± 0.08	1,105.73 ± 0.10	1,105.60 ± 0.04	1,105.41 ± 0.09	1,105.30 ± 0.50
1,083.91 ± 0.09	1,082.82 ± 0.09	1,082.89 ± 0.04	1,083.10 ± 0.08	1,083.11 ± 0.13	1,083.88 ± 0.24	1,079.52 ± 0.34	1,079.54 ± 0.16	1,079.48 ± 0.17	1,079.40 ± 0.14	1,079.63 ± 0.22

¹Values are means ± SE of 3 replicates.

Table 3. Wavenumbers (cm⁻¹) of distinct peaks of *Lactobacillus acidophilus* as affected by varying NaCl concentrations¹

NaCl (% wt/vol)										
0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0
3,285.56 ± 0.25	3,285.81 ± 0.29	3,285.62 ± 0.28	3,285.04 ± 0.34	3,285.64 ± 0.68	3,284.17 ± 0.05	3,287.08 ± 2.00	3,287.07 ± 0.72	3,291.08 ± 0.83	3,291.62 ± 0.30	3,292.06 ± 0.47
2,962.62 ± 0.59	2,962.24 ± 0.58	2,962.10 ± 0.23	2,961.67 ± 0.17	2,961.75 ± 0.19	2,962.46 ± 0.22	2,962.16 ± 0.02	2,962.16 ± 0.11	2,966.32 ± 3.93	2,961.78 ± 0.05	2,961.98 ± 0.02
2,926.10 ± 0.52	2,925.93 ± 0.38	2,925.43 ± 0.31	2,932.69 ± 0.14	2,924.90 ± 0.07	2,927.16 ± 0.64	2,926.65 ± 0.99	2,926.11 ± 0.66	2,930.38 ± 1.10	2,929.45 ± 1.76	2,931.43 ± 1.44
2,853.43 ± 0.12	2,853.28 ± 0.04	2,853.32 ± 0.04	2,852.87 ± 0.04	2,853.24 ± 0.01	2,853.29 ± 0.04	2,853.23 ± 0.08	2,853.23 ± 0.10	2,853.35 ± 0.03	2,853.34 ± 0.05	
1,661.99 ± 0.64	1,662.54 ± 1.50	1,664.22 ± 0.92	1,660.63 ± 1.06	1,662.46 ± 1.58	1,660.26 ± 0.06	1,661.45 ± 1.72	1,659.70 ± 2.61	1,667.54 ± 0.29	1,667.99 ± 0.47	
1,648.61 ± 1.32	1,648.79 ± 1.00	1,646.96 ± 0.40	1,650.41 ± 0.17	1,647.16 ± 1.06	1,652.49 ± 1.12	1,650.82 ± 1.55	1,650.22 ± 0.76	1,653.74 ± 2.09	1,653.17 ± 2.10	1,653.26 ± 1.42
1,634.13 ± 0.78	1,633.52 ± 0.40	1,633.10 ± 0.04	1,635.42 ± 0.78	1,634.27 ± 1.16	1,635.25 ± 1.03	1,634.51 ± 0.88	1,635.25 ± 1.05	1,636.64 ± 0.46	1,636.13 ± 0.53	1,636.29 ± 0.16
1,552.41 ± 0.14	1,553.08 ± 0.29	1,553.03 ± 0.38	1,553.56 ± 0.21	1,552.14 ± 1.19	1,551.03 ± 0.01	1,551.63 ± 0.82	1,554.62 ± 2.73	1,557.15 ± 0.28	1,557.28 ± 0.13	1,557.36 ± 0.31
1,453.77 ± 0.08	1,454.31 ± 0.24	1,453.86 ± 0.23	1,454.65 ± 0.10	1,453.93 ± 0.37	1,454.65 ± 0.66	1,454.50 ± 0.71	1,454.82 ± 0.64	1,455.38 ± 0.52	1,455.41 ± 0.54	1,455.63 ± 0.41
1,241.03 ± 0.10	1,241.30 ± 0.14	1,240.77 ± 0.31	1,241.30 ± 0.15	1,240.69 ± 0.40	1,240.62 ± 0.64	1,240.22 ± 0.58	1,240.07 ± 0.71	1,240.21 ± 0.93	1,240.19 ± 0.91	1,240.19 ± 0.67
1,124.81 ± 0.38	1,125.44 ± 0.08	1,125.04 ± 0.20	1,125.33 ± 0.10	1,125.26 ± 0.12	1,125.31 ± 0.18	1,125.62 ± 0.10	1,125.60 ± 0.08	1,125.75 ± 0.06	1,125.80 ± 0.16	1,126.18 ± 0.07
1,082.61 ± 0.23	1,082.71 ± 0.11	1,082.58 ± 0.12	1,082.09 ± 0.12	1,082.51 ± 0.24	1,081.57 ± 0.27	1,081.77 ± 0.24	1,081.97 ± 0.08	1,081.36 ± 0.38	1,081.83 ± 0.16	1,081.99 ± 0.05

¹Values are means ± SE of 3 replicates.

Table 4. Wavenumbers (cm⁻¹) of distinct peaks of *Lactobacillus casei* as affected by varying NaCl concentrations¹

NaCl (% wt/vol)										
0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0
3,286.26 ± 0.23	3,286.52 ± 0.11	3,286.27 ± 0.03	3,286.16 ± 0.23	3,286.16 ± 0.26	3,286.17 ± 0.04	3,286.37 ± 0.24	3,286.19 ± 0.13	3,286.55 ± 0.31	3,286.58 ± 0.18	3,286.25 ± 0.58
2,962.14 ± 0.21	2,962.04 ± 0.06	2,962.20 ± 0.14	2,962.18 ± 0.05	2,962.12 ± 0.10	2,962.27 ± 0.31	2,962.22 ± 0.16	2,962.31 ± 0.09	2,962.38 ± 0.38	2,962.36 ± 0.27	2,962.35 ± 0.12
2,925.72 ± 0.33	2,925.60 ± 0.16	2,926.24 ± 0.25	2,925.36 ± 0.07	2,926.08 ± 0.20	2,926.77 ± 0.20	2,926.57 ± 0.19	2,926.64 ± 0.15	2,927.06 ± 0.40	2,927.31 ± 0.73	2,926.91 ± 0.53
2,853.39 ± 0.03	2,853.35 ± 0.05	2,853.44 ± 0.03	2,853.16 ± 0.05	2,853.36 ± 0.03	2,853.42 ± 0.02	2,853.42 ± 0.01	2,853.39 ± 0.01	2,853.43 ± 0.02	2,853.42 ± 0.01	2,853.29 ± 0.01
1,665.17 ± 0.37	1,664.08 ± 1.48	1,665.55 ± 0.20	1,665.07 ± 0.61	1,664.42 ± 1.31	1,665.09 ± 0.29	1,665.08 ± 0.28	1,664.58 ± 0.49	1,664.81 ± 0.13	1,664.05 ± 0.69	1,663.23 ± 2.06
1,646.99 ± 0.32	1,648.04 ± 1.07	1,647.21 ± 0.48	1,646.93 ± 0.31	1,647.62 ± 0.61	1,647.14 ± 0.44	1,647.27 ± 0.46	1,647.54 ± 0.41	1,647.34 ± 0.60	1,647.83 ± 1.01	1,648.09 ± 0.46
1,631.78 ± 0.21	1,633.17 ± 0.58	1,631.13 ± 0.21	1,632.97 ± 0.47	1,632.16 ± 0.83	1,631.44 ± 0.47	1,631.26 ± 0.24	1,631.64 ± 0.36	1,632.38 ± 0.73	1,633.05 ± 1.02	1,633.85 ± 2.11
1,553.48 ± 0.04	1,553.27 ± 0.25	1,553.76 ± 0.20	1,553.22 ± 0.21	1,553.20 ± 0.55	1,553.34 ± 0.20	1,553.33 ± 0.19	1,553.29 ± 0.47	1,553.30 ± 0.46	1,553.33 ± 0.56	1,552.70 ± 1.25
1,453.25 ± 0.27	1,454.33 ± 0.25	1,453.52 ± 0.23	1,453.97 ± 0.09	1,453.88 ± 0.19	1,453.48 ± 0.24	1,453.51 ± 0.28	1,453.81 ± 0.21	1,453.83 ± 0.37	1,453.94 ± 0.48	1,453.99 ± 0.32
1,242.13 ± 0.09	1,241.75 ± 0.15	1,241.08 ± 0.24	1,241.71 ± 0.01	1,241.14 ± 0.36	1,240.50 ± 0.66	1,240.29 ± 0.41	1,240.11 ± 0.51	1,240.45 ± 0.74	1,240.58 ± 0.59	1,240.54 ± 0.60
1,123.08 ± 0.28	1,123.01 ± 0.19	1,122.89 ± 0.40	1,123.09 ± 0.17	1,122.95 ± 0.39	1,122.25 ± 0.49	1,122.62 ± 0.47	1,122.66 ± 0.40	1,122.15 ± 0.63	1,122.47 ± 0.59	1,122.26 ± 0.56
1,082.49 ± 0.56	1,082.36 ± 0.33	1,081.94 ± 0.70	1,082.14 ± 0.18	1,082.30 ± 0.59	1,080.48 ± 1.25	1,080.57 ± 1.12	1,080.79 ± 1.13	1,080.42 ± 1.13	1,080.62 ± 1.27	1,082.09 ± 0.49

¹Values are means ± SE of 3 replicates.

Table 5. Wavenumbers (cm^{-1}) of distinct peaks of *Escherichia coli* as affected by varying NaCl concentrations^{1,2}

NaCl (% wt/vol)												
0	0.5	1.0	1.5	2.0	2.5	3.0	3.5					
3,284.70 ± 0.04	3,283.74 ± 0.90	3,284.87 ± 0.12	3,282.70 ± 0.71	3,285.40 ± 0.60	3,283.31 ± 0.13	3,284.45 ± 0.38	3,278.41 ± 0.41					
2,961.20 ± 0.03	2,961.30 ± 0.08	2,961.38 ± 0.10	2,961.18 ± 0.01	2,961.45 ± 0.05	2,960.93 ± 0.04	2,961.08 ± 0.04	2,960.30 ± 0.07					
2,922.86 ± 0.09	2,922.64 ± 0.18	2,922.46 ± 0.13	2,922.52 ± 0.17	2,922.62 ± 0.20	2,923.04 ± 0.10	2,923.13 ± 0.28	2,922.64 ± 0.13					
2,852.63 ± 0.02	2,852.41 ± 0.10	2,852.40 ± 0.09	2,852.39 ± 0.10	2,852.51 ± 0.05	2,852.58 ± 0.04	2,852.66 ± 0.14	2,852.38 ± 0.10					
1,663.04 ± 1.20	1,662.78 ± 0.51	1,661.79 ± 0.68	1,660.69 ± 0.43	1,664.10 ± 2.01	1,662.49 ± 2.33	1,661.15 ± 1.48	1,652.31 ± 0.25					
1,647.26 ± 1.75	1,651.88 ± 1.79	1,648.28 ± 1.76	1,652.38 ± 0.74	1,646.22 ± 1.47	1,653.73 ± 0.14	1,647.70 ± 2.68	1,633.66 ± 0.64					
1,631.73 ± 0.61	1,635.12 ± 1.51	1,631.75 ± 0.55	1,633.33 ± 0.76	1,630.81 ± 0.30	1,632.04 ± 0.19	1,630.69 ± 0.68	1,555.99 ± 0.31					
1,552.54 ± 0.90	1,553.86 ± 0.03	1,552.54 ± 0.89	1,554.17 ± 0.24	1,552.74 ± 1.30	1,551.68 ± 0.48	1,552.35 ± 1.26	1,456.14 ± 0.14					
1,452.13 ± 2.20	1,455.29 ± 0.33	1,452.24 ± 2.11	1,455.32 ± 0.25	1,452.00 ± 2.36	1,454.44 ± 0.07	1,452.07 ± 2.74	1,241.74 ± 0.26					
1,242.03 ± 0.15	1,242.28 ± 0.06	1,242.25 ± 0.06	1,242.08 ± 0.05	1,241.96 ± 0.50	1,241.13 ± 0.22	1,241.38 ± 0.78	1,084.10 ± 0.16					
1,120.62 ± 0.07	1,120.56 ± 0.10	1,120.51 ± 0.27	1,120.60 ± 0.08	1,120.80 ± 0.19	1,113.22 ± 7.73	1,119.53 ± 0.59						
1,082.53 ± 0.05	1,083.29 ± 0.17	1,083.82 ± 0.30	1,083.81 ± 0.03	1,084.30 ± 0.14	1,083.77 ± 0.20	1,084.20 ± 0.06						

¹Peaks at NaCl concentrations of 4.0, 4.5, and 5.0% were not determined.²Values are means ± SE of 3 replicates.

phimurium' and 'Enteritidis'. The amide-I and amide-II regions showed higher sensitivity to the NaCl concentration and the most prominent changes were observed in the peaks around $1,647 \text{ cm}^{-1}$, which are associated with the irregular structures. No substantial shift in the peak frequency was observed for the amide-III region of *E. coli* upon altering the salt concentration.

The effect of NaCl concentration was different for different bacteria; however, for most of the bacteria, a change was observed in the amide-I region with the increasing NaCl concentration. Different structural modifications were observed for different bacteria used in this study and *Lb. casei* appeared to be the least affected by varying NaCl concentrations. The findings from FTIR spectroscopy suggest that although the other bacteria were affected by NaCl, the stability of membrane fluidity was retained in most bacteria at least up to 2.5% NaCl concentration. These results corroborate the findings from the cell count, suggesting 2.5% NaCl as the critical concentration.

Effect of NaCl Substitution with KCl on FTIR Spectra. The FTIR spectra for each bacterium as recorded at varying concentrations of KCl are shown in Tables 6 to 10. The FTIR spectra of *Lc. lactis* ssp. *lactis* (Table 6) at varying substitution levels suggested stability of most of the surface functional groups. The amide-A group ($4,000$ to $3,100 \text{ cm}^{-1}$) with a peak at $3,284 \text{ cm}^{-1}$ and the amide-II region ($\sim 1,550 \text{ cm}^{-1}$), was unaffected ($P < 0.05$) by substitution of NaCl with KCl. The peak at approximately $3,300 \text{ cm}^{-1}$ is associated with N-H stretching modes of the amide-A region. Similarly, the stretching in CH_3 and CH_2 bands associated with the membrane FA ($3,000$ to $2,800 \text{ cm}^{-1}$) showed no shifts upon varying the NaCl-to-KCl ratio. The CH_2 symmetric stretching band located at $2,853 \text{ cm}^{-1}$ is associated with membrane fluidity, where an increase in the peak frequency implies increased membrane fluidity. Substitution with KCl did not affect the membrane fluidity of *Lc. lactis* ssp. *lactis*, as indicated by stable peak frequency at various KCl substitution levels. However, certain changes were observed in the amide-I region ($1,700$ – $1,600 \text{ cm}^{-1}$) of *Lc. lactis* ssp. *lactis*, particularly those attributed to the irregular structures. A gradual increase in the wavenumber was observed at $1,660 \text{ cm}^{-1}$, associated with the random coils, as the amount of KCl was increased. Also, the band at $1,641 \text{ cm}^{-1}$, assigned to irregular protein structures, increased significantly ($P < 0.05$), at low levels of KCl substitution as well. The peak attributed to β -sheets ($1,240 \text{ cm}^{-1}$) as per the tentative assignment by Cai and Singh (1999), also showed stability with varying substitution levels.

Among the probiotic bacteria examined in this study, *B. longum* revealed spectral changes (Table 7) in all

Table 6. Fourier-transform infrared spectra (cm^{-1}) for *Lactococcus lactis* ssp. *lactis* at varying levels of NaCl substitution with KCl, at 2.5% total salt concentration¹

KCl (% of total salt)				
0	25	50	75	100
3,284.79 $\pm$ 0.43	3,285.31 $\pm$ 0.71	3,285.22 $\pm$ 0.71	3,284.91 $\pm$ 0.67	3,285.44 $\pm$ 0.56
2,961.85 $\pm$ 0.05	2,961.86 $\pm$ 0.02	2,961.83 $\pm$ 0.06	2,961.93 $\pm$ 0.04	2,961.89 $\pm$ 0.03
2,925.72 $\pm$ 0.13	2,925.57 $\pm$ 0.10	2,925.54 $\pm$ 0.07	2,925.30 $\pm$ 0.31	2,925.15 $\pm$ 0.27
2,853.41 $\pm$ 0.02	2,853.43 $\pm$ 0.04	2,853.43 $\pm$ 0.02	2,853.45 $\pm$ 0.03	2,853.38 $\pm$ 0.03
1,659.84 $\pm$ 1.53	1,661.45 $\pm$ 2.37	1,661.44 $\pm$ 2.34	1,661.86 $\pm$ 2.28	1,661.19 $\pm$ 2.44
1,641.31 $\pm$ 2.09	1,646.04 $\pm$ 0.07	1,645.75 $\pm$ 0.22	1,645.85 $\pm$ 0.33	
	1,634.97 $\pm$ 2.15	1,635.63 $\pm$ 2.16	1,635.73 $\pm$ 2.20	1,636.32 $\pm$ 2.40
1,550.33 $\pm$ 0.62	1,551.23 $\pm$ 1.12	1,550.85 $\pm$ 1.35	1,551.68 $\pm$ 1.15	1,551.10 $\pm$ 1.23
1,453.08 $\pm$ 0.07	1,453.25 $\pm$ 0.25	1,453.45 $\pm$ 0.05	1,453.37 $\pm$ 0.10	1,453.14 $\pm$ 0.16
1,240.42 $\pm$ 0.11	1,240.26 $\pm$ 0.07	1,240.46 $\pm$ 0.14	1,240.70 $\pm$ 0.08	1,240.42 $\pm$ 0.04
1,122.58 $\pm$ 0.02	1,122.70 $\pm$ 0.02	1,122.29 $\pm$ 0.24	1,122.30 $\pm$ 0.28	1,122.30 $\pm$ 0.14
1,079.96 $\pm$ 0.4	1,080.11 $\pm$ 0.12	1,080.32 $\pm$ 0.50	1,080.70 $\pm$ 0.62	1,081.07 $\pm$ 0.40

¹Values are means $\pm$ SE of 3 replicates.

the amide regions. A significant ($P < 0.05$) shift from 3,288 (0% KCl) to 3,285 cm^{-1} (25% KCl) was observed in the amide-A region when NaCl was substituted with KCl. This is indicative of the shortening of the hydrogen bond in the peptide backbone (Krimm and Bandekar, 1986). The amide-I region at approximately 1,652 cm^{-1} , attributed to α -helices, showed an increase in the wavenumber in 100% KCl compared with the control (0% KCl); however, not much change was observed in the other substitution levels. The amide-II and the amide-III regions showed greater stability in wavenumber, even with an increase in the amount of NaCl substituted.

The FTIR spectra of *Lb. acidophilus* as affected by substitution of NaCl with KCl are depicted in Table 8. A significant decrease ($P < 0.05$) in the wavenumber was observed upon substituting 75% NaCl with KCl in the amide-A region (3,285 cm^{-1}); however, at 100% substitution, the peak frequency was same as that at

0% substitution. This could imply that the presence of both NaCl and KCl caused a shift in the amide-A region. In the amide-III region (1,240 cm^{-1}), a gradual increase was observed in the wavenumber upon increasing the substitution with KCl, which was maximum at 75% KCl. The spectra indicate that substitution of NaCl up to 50% may not affect the surface functional groups considerably. However, substitution beyond 50% NaCl may cause shifts in the amide regions of *Lb. acidophilus*.

Lactobacillus casei was the least affected probiotic bacterium, as indicated by analysis of the FTIR spectra (Table 9). Most of the regions were unaffected by KCl substitution, except for the amide-I region. A gradual shift from 1,647 cm^{-1} (at 0% KCl) to 1,648 cm^{-1} (at 50% KCl) upon increasing the substitution level is indicative of the changes occurring in the irregular structures toward α -helices in *Lb. casei* (Kilimann et al., 2006). The region 1,652 to 1,648 cm^{-1} is assigned to

Table 7. Wavenumbers (cm^{-1}) for distinct peaks of *Bifidobacterium longum* at varying levels of NaCl substitution with KCl, at 2.5% total salt concentration¹

KCl (% of total salt)				
0	25	50	75	100
3,288.23 $\pm$ 1.69	3,285.79 $\pm$ 0.33	3,286.13 $\pm$ 0.31	3,285.49 $\pm$ 0.55	3,285.72 $\pm$ 0.12
2,960.63 $\pm$ 0.06	2,960.92 $\pm$ 0.13	2,961.26 $\pm$ 0.09	2,961.39 $\pm$ 0.09	2,961.48 $\pm$ 0.06
2,923.47 $\pm$ 0.37	2,924.05 $\pm$ 0.11	2,924.22 $\pm$ 0.09	2,924.30 $\pm$ 0.05	2,924.38 $\pm$ 0.06
2,852.44 $\pm$ 0.26	2,852.77 $\pm$ 0.07	2,852.94 $\pm$ 0.04	2,852.91 $\pm$ 0.03	2,852.87 $\pm$ 0.02
1,660.70 $\pm$ 0.12	1,660.74 $\pm$ 0.21	1,662.32 $\pm$ 1.61	1,660.90 $\pm$ 0.18	1,659.47 $\pm$ 0.84
1,650.88 $\pm$ 0.31	1,652.63 $\pm$ 0.89	1,650.08 $\pm$ 1.90	1,651.64 $\pm$ 1.09	1,652.51 $\pm$ 0.97
1,633.62 $\pm$ 1.41	1,634.96 $\pm$ 1.60	1,632.72 $\pm$ 0.87	1,633.69 $\pm$ 1.57	1,634.95 $\pm$ 1.09
1,554.38 $\pm$ 1.13	1,553.43 $\pm$ 0.57	1,553.10 $\pm$ 0.50	1,553.04 $\pm$ 0.40	1,552.61 $\pm$ 0.11
1,455.09 $\pm$ 0.60	1,454.96 $\pm$ 0.50	1,454.54 $\pm$ 0.30	1,454.71 $\pm$ 0.36	1,454.89 $\pm$ 0.25
1,239.79 $\pm$ 0.67	1,240.40 $\pm$ 0.37	1,241.02 $\pm$ 0.12	1,241.02 $\pm$ 0.09	1,240.63 $\pm$ 0.17
1,106.12 $\pm$ 0.21	1,106.49 $\pm$ 0.16	1,107.01 $\pm$ 0.09	1,107.05 $\pm$ 0.10	1,107.11 $\pm$ 0.08
1,079.84 $\pm$ 0.39	1,080.10 $\pm$ 0.21	1,080.66 $\pm$ 0.15	1,080.84 $\pm$ 0.10	1,080.88 $\pm$ 0.06

¹Values are means $\pm$ SE of 3 replicates.

Table 8. Wavenumbers (cm^{-1}) for distinct peaks of *Lactobacillus acidophilus* at varying levels of NaCl substitution with KCl, at 2.5% total salt concentration¹

KCl (% of total salt)				
0	25	50	75	100
3,285.02 $\pm$ 0.45	3,284.56 $\pm$ 0.46	3,284.93 $\pm$ 0.24	3,283.83 $\pm$ 0.20	3,285.04 $\pm$ 0.24
2,961.33 $\pm$ 0.08	2,961.53 $\pm$ 0.19	2,961.71 $\pm$ 0.09	2,961.69 $\pm$ 0.12	2,961.49 $\pm$ 0.15
2,924.44 $\pm$ 0.17	2,924.60 $\pm$ 0.43	2,925.16 $\pm$ 0.23	2,925.31 $\pm$ 0.19	2,924.63 $\pm$ 0.20
2,853.00 $\pm$ 0.10	2,853.02 $\pm$ 0.07	2,853.15 $\pm$ 0.04	2,853.15 $\pm$ 0.09	2,853.04 $\pm$ 0.09
1,659.38 $\pm$ 2.70	1,659.13 $\pm$ 2.73	1,660.10 $\pm$ 1.74	1,657.74 $\pm$ 0.76	1,658.81 $\pm$ 1.43
1,635.86 $\pm$ 2.07	1,636.00 $\pm$ 2.07	1,635.58 $\pm$ 1.79	1,638.56 $\pm$ 0.14	1,635.36 $\pm$ 1.77
1,549.51 $\pm$ 1.75	1,549.41 $\pm$ 2.78	1,551.89 $\pm$ 0.81	1,547.84 $\pm$ 1.23	1,549.55 $\pm$ 1.66
1,453.48 $\pm$ 0.64	1,453.88 $\pm$ 0.48	1,453.57 $\pm$ 0.12	1,453.61 $\pm$ 0.08	1,453.68 $\pm$ 0.34
1,239.64 $\pm$ 0.99	1,240.14 $\pm$ 0.66	1,240.42 $\pm$ 0.27	1,240.70 $\pm$ 0.28	1,240.55 $\pm$ 0.53
1,125.34 $\pm$ 0.20	1,124.95 $\pm$ 0.28	1,124.96 $\pm$ 0.19	1,125.14 $\pm$ 0.21	1,125.06 $\pm$ 0.17
1,081.97 $\pm$ 0.30	1,081.98 $\pm$ 0.16	1,082.09 $\pm$ 0.20	1,081.91 $\pm$ 0.13	1,082.22 $\pm$ 0.27

¹Values are means $\pm$ SE of 3 replicates.

α -helices, and the region 1,648 to 1,642 cm^{-1} is assigned to irregular structures (Hussain et al., 2011).

Escherichia coli indicated the sensitivity of amide regions upon substituting 50% NaCl with KCl (Table 10). A gradual decrease in the peak frequency was observed in the amide-A region (3,284 cm^{-1}). The FA region was stable, with no change in the membrane fluidity, as indicated by the stable peak frequency at 2,852 cm^{-1} . Significant decline in the wavenumbers was observed in the amide-I region from 1,661 and 1,632 cm^{-1} at 0% KCl compared with 1,656 and 1,636 cm^{-1} in 100% substitution with KCl.

The substitution of NaCl with KCl had little effect on most of the bacteria, as reflected by the FTIR spectroscopic analysis. Certain changes in the amide regions were observed; however, the membrane fluidity and the FA region showed stability upon substitution with KCl for most bacteria. This is also supported by the cell viability results that indicated that low substi-

tution levels (0 and 25%) did not significantly affect most of the bacteria, whereas *Lc. lactis* ssp. *lactis* as and *Lb. acidophilus* also had no significant effect at higher substitution levels. Membrane lipids were found to be affected by different cations differently; a weak binding of Na cations to membrane lipids was observed, in contrast to no cation binding in the case of KCl (Gurtovenko and Vattulainen, 2008).

Sodium chloride induced changes in the cell membrane and affected cell viability in all bacteria. The extent of changes was found to be bacteria dependent. Although most studies postulate that the mechanism of NaCl-induced damage to bacteria is due to plasmolysis, several others have shown that the effect is caused mainly by electrostatic contraction of amphoteric and polyionic cell wall polymers. Fourier-transform infrared spectroscopic study has revealed that significant changes occur in the bacterial cell membrane when subjected to varying concentrations of NaCl and KCl.

Table 9. Wavenumbers (cm^{-1}) for distinct peaks of *Lactobacillus casei* at varying levels of NaCl substitution with KCl, at 2.5% total salt concentration¹

KCl (% of total salt)				
0	25	50	75	100
3,285.30 $\pm$ 0.41	3,286.40 $\pm$ 0.65	3,285.40 $\pm$ 0.67	3,286.32 $\pm$ 0.14	3,286.21 $\pm$ 0.14
2,962.02 $\pm$ 0.04	2,962.14 $\pm$ 0.01	2,962.03 $\pm$ 0.08	2,961.99 $\pm$ 0.11	2,961.98 $\pm$ 0.04
2,926.46 $\pm$ 0.17	2,926.16 $\pm$ 0.20	2,926.45 $\pm$ 0.22	2,925.69 $\pm$ 0.36	2,926.43 $\pm$ 0.09
2,853.42 $\pm$ 0.02	2,853.42 $\pm$ 0.01	2,853.42 $\pm$ 0.02	2,853.38 $\pm$ 0.02	2,853.43 $\pm$ 0.01
1,664.09 $\pm$ 1.10	1,666.06 $\pm$ 0.18	1,664.10 $\pm$ 1.78	1,666.21 $\pm$ 0.11	1,665.83 $\pm$ 0.16
1,647.92 $\pm$ 1.24	1,646.86 $\pm$ 0.38	1,648.58 $\pm$ 1.55	1,647.08 $\pm$ 0.13	1,647.07 $\pm$ 0.07
1,631.24 $\pm$ 0.43	1,631.19 $\pm$ 0.20	1,630.58 $\pm$ 0.26	1,631.22 $\pm$ 0.30	1,630.94 $\pm$ 0.45
1,552.89 $\pm$ 0.39	1,553.71 $\pm$ 0.27	1,552.85 $\pm$ 0.71	1,554.00 $\pm$ 0.17	1,553.45 $\pm$ 0.20
1,453.85 $\pm$ 0.20	1,453.70 $\pm$ 0.19	1,453.70 $\pm$ 0.11	1,453.93 $\pm$ 0.07	1,453.95 $\pm$ 0.10
1,240.10 $\pm$ 0.20	1,239.86 $\pm$ 0.35	1,239.51 $\pm$ 0.17	1,239.60 $\pm$ 0.27	1,239.84 $\pm$ 0.31
1,122.12 $\pm$ 0.13	1,122.84 $\pm$ 0.24	1,122.32 $\pm$ 0.43	1,122.66 $\pm$ 0.54	1,121.97 $\pm$ 0.24
1,081.44 $\pm$ 0.46	1,081.67 $\pm$ 0.17	1,081.30 $\pm$ 0.38	1,081.92 $\pm$ 0.68	1,081.64 $\pm$ 0.22

¹Values are means $\pm$ SE of 3 replicates.

Table 10. Wavenumbers (cm^{-1}) for distinct peaks of *Escherichia coli* at varying levels of NaCl substitution with KCl, at 2.5% total salt concentration¹

KCl (% of total salt)				
0	25	50	75	100
3,284.71 $\pm$ 0.22	3,284.32 $\pm$ 0.04	3,283.77 $\pm$ 0.12	3,283.81 $\pm$ 0.10	3,283.85 $\pm$ 0.12
2,961.20 $\pm$ 0.09	2,961.20 $\pm$ 0.09	2,961.15 $\pm$ 0.09	2,961.14 $\pm$ 0.07	2,961.10 $\pm$ 0.08
2,923.19 $\pm$ 0.05	2,923.09 $\pm$ 0.06	2,923.07 $\pm$ 0.04	2,922.89 $\pm$ 0.05	2,923.05 $\pm$ 0.05
2,852.71 $\pm$ 0.03	2,852.63 $\pm$ 0.02	2,852.61 $\pm$ 0.02	2,852.52 $\pm$ 0.03	2,852.61 $\pm$ 0.03
1,661.80 $\pm$ 1.61	1,659.37 $\pm$ 0.47	1,657.59 $\pm$ 0.39	1,655.69 $\pm$ 0.60	1,656.69 $\pm$ 0.72
1,650.47 $\pm$ 1.80				
1,632.12 $\pm$ 0.43	1,633.46 $\pm$ 0.66	1,635.28 $\pm$ 0.85	1,636.59 $\pm$ 0.71	1,636.65 $\pm$ 0.83
1,551.64 $\pm$ 0.42	1,551.00 $\pm$ 0.27	1,549.91 $\pm$ 0.28	1,549.12 $\pm$ 0.49	1,545.55 $\pm$ 1.64
1,453.54 $\pm$ 0.06	1,453.73 $\pm$ 0.04	1,453.93 $\pm$ 0.01	1,454.14 $\pm$ 0.05	1,454.07 $\pm$ 0.05
1,242.35 $\pm$ 0.06	1,242.17 $\pm$ 0.11	1,242.04 $\pm$ 0.16	1,241.93 $\pm$ 0.07	1,242.01 $\pm$ 0.02
1,120.30 $\pm$ 0.37	1,120.38 $\pm$ 0.37	1,120.30 $\pm$ 0.54	1,120.27 $\pm$ 0.20	1,119.98 $\pm$ 0.24
1,084.45 $\pm$ 0.13	1,084.32 $\pm$ 0.15	1,084.11 $\pm$ 0.08	1,084.09 $\pm$ 0.10	1,083.73 $\pm$ 0.06

¹Values are means $\pm$ SE of 3 replicates.

CONCLUSIONS

Reduction of NaCl to the critical NaCl concentration, which inhibited *E. coli* without significantly affecting the viability of the probiotic bacteria, was determined as 2.5% (wt/vol). At this concentration, the viability of *E. coli* was not significantly higher than at 3.0%; however, the viability of *B. longum* was significantly more than at 3.0% NaCl. This is also supported by the FTIR spectroscopic analysis, where significant changes were observed in most of the bacteria upon increasing the concentration from 2.5 to 3.0% NaCl. Substitution of NaCl with KCl up to 50% of the total salt could be considered as optimum, at 2.5% total salt, to control the viability of *E. coli*. Fourier-transform infrared spectroscopic study indicates that *Lc. lactis* ssp. *lactis* and the 2 lactobacilli are relatively more stable to the varying KCl substitution, compared with *B. longum* and *E. coli*. It would be interesting to further investigate the cause-effect relationship of NaCl reduction and substitution by KCl on bacterial structure and functions in a food matrix.

REFERENCES

- Albarracin, W., I. C. Sánchez, R. Grau, and J. M. Barat. 2011. Salt in food processing; usage and reduction: A review. *Int. J. Food Sci. Technol.* 46:1329–1336.
- Altenbach, C., and J. Seelig. 1984. Ca^{2+} binding to phosphatidylcholine bilayers as studied by deuterium magnetic-resonance. Evidence for the formation of a Ca^{2+} complex with 2 phospholipid molecules. *Biochemistry* 23:3913–3920.
- Alvarez-Ordóñez, A., J. Halisch, and M. Prieto. 2010. Changes in Fourier transform infrared spectra of *Salmonella enterica* serovars Typhimurium and Enteritidis after adaptation to stressful growth conditions. *Int. J. Food Microbiol.* 142:97–105.
- Ayyash, M. M., and N. P. Shah. 2011a. Effect of partial substitution of NaCl with KCl on proteolysis of Halloumi cheese. *J. Food Sci.* 76:C31–C37.
- Ayyash, M. M., and N. P. Shah. 2011b. The effect of substituting NaCl with KCl on Nabulsi cheese: Chemical composition, total viable count, and texture profile. *J. Dairy Sci.* 94:2741–2751.
- Beattie, S. H., C. Holt, D. Hirst, and A. G. Williams. 1998. Discrimination among *Bacillus cereus*, *B. mycoides* and *B. thuringiensis* and some other species of the genus *Bacillus* by Fourier transform infrared spectroscopy. *FEMS Microbiol. Lett.* 164:201–206.
- Beney, L., and P. Gervais. 2001. Influence of the fluidity of the membrane on the response of microorganisms to environmental stresses. *Appl. Microbiol. Biotechnol.* 57:34–42.
- Beresford, T. P., N. A. Fitzsimons, N. L. Brennan, and T. M. Cogan. 2001. Recent advances in cheese microbiology. *Int. Dairy J.* 11:259–274.
- Binder, H., and O. Zschörnig. 2002. The effect of metal cations on the phase behavior and hydration characteristics of phospholipid membranes. *Chem. Phys. Lipids* 115:39–61.
- Buemi, M., M. Senatore, F. Corica, C. Aloisi, A. Romeo, D. Tramontana, and N. Frisina. 2002. Diet and arterial hypertension: Is the sodium ion alone important? *Med. Res. Rev.* 22:419–428.
- Cai, S., and B. R. Singh. 1999. Identification of β -turn and random coil amide III infrared bands for secondary structure estimation of proteins. *Biophys. Chem.* 80:7–20.
- Cai, S., and B. R. Singh. 2004. A distinct utility of the amide III infrared band for secondary structure estimation of aqueous protein solutions using partial least squares methods. *Biochemistry* 43:2541–2549.
- Cruz, A. G., J. A. F. Faria, M. A. R. Pollonio, H. M. A. Bolini, R. M. S. Celeghini, D. Granato, and N. P. Shah. 2011. Cheeses with reduced sodium content: Effects on functionality, public health benefits and sensory properties. *Trends Food Sci. Technol.* 22:276–291.
- Fitzgerald, E., and J. Buckley. 1985. Effect of total and partial substitution of sodium chloride on the quality of Cheddar cheese. *J. Dairy Sci.* 68:3127–3134.
- Fukuma, T., M. J. Higgins, and S. P. Jarvis. 2007. Direct imaging of lipid-ion network formation under physiological conditions by frequency modulation atomic force microscopy. *Phys. Rev. Lett.* 98:106101.
- Goodridge, L., J. Chen, and M. Griffiths. 1999. The use of a fluorescent bacteriophage assay for detection of *Escherichia coli* O157:H7 in inoculated ground beef and raw milk. *Int. J. Food Microbiol.* 47:43–50.
- Gurtovenko, A. A., and I. Vattulainen. 2008. Effect of NaCl and KCl on phosphatidylcholine and phosphatidylethanolamine lipid membranes: Insight from atomic-scale simulations for understanding salt-induced effects in the plasma membrane. *J. Phys. Chem. B* 112:1953–1962.
- Heaney, R. P. 2006. Role of dietary sodium in osteoporosis. *J. Am. Coll. Nutr.* 25(Suppl.):271S–276S.
- Herbette, L., C. A. Napolitano, and R. V. McDaniel. 1984. Direct determination of the calcium profile structure for dipalmitoyllecithin multilayers using neutron diffraction. *Biophys. J.* 46:677–685.

- Hussain, R., C. Gaiani, L. Aberkane, J. Ghanbaja, and J. Scher. 2011. Multiscale characterization of casein micelles under NaCl range conditions. *Food Biophys.* 6:503–511.
- Kilimann, K. V., W. Doster, R. F. Vogel, C. Hartmann, and M. G. Gänzle. 2006. Protection by sucrose against heat-induced lethal and sublethal injury of *Lactococcus lactis*: An FT-IR study. *Biochim. Biophys. Acta* 1764:1188–1197.
- Kotchen, T. A. 2005. Contributions of sodium and chloride to NaCl-induced hypertension. *Hypertension* 45:849–850.
- Krimm, S., and J. Bandekar. 1986. Vibrational spectroscopy and conformation of peptides, polypeptides, and proteins. *Adv. Protein Chem.* 38:181–364.
- Massey, L. K. 2005. Effect of dietary salt intake on circadian calcium metabolism, bone turnover, and calcium oxalate kidney stone risk in postmenopausal women. *Nutr. Res.* 25:891–903.
- Naumann, D., D. Helm, H. Labischinski, and P. Giesbrecht. 1991. The characterization of microorganisms by Fourier-transform infrared spectroscopy (FT-IR). Pages 43–96 in *Modern Techniques for Rapid Microbiological Analysis*. W. H. Nelson, ed. VCH Publishers Inc., New York, NY.
- Nielsen, H.-J. S., and P. Zeuthen. 1987. Microbiological effects of a partial or total replacement of sodium in sodium chloride with other cations—Model systems. *Int. J. Food Microbiol.* 4:13–24.
- Prasad, J., P. McJarrow, and P. Gopal. 2003. Heat and osmotic stress responses of probiotic *Lactobacillus rhamnosus* HN001 (DR20) in relation to viability after drying. *Appl. Environ. Microbiol.* 69:917–925.
- Roy, D. 1991. Salt stress on growth and acid production of *Lactobacillus helveticus* strain milano. *Lett. Appl. Microbiol.* 12:207–211.
- Santivarangkna, C., M. Wenning, P. Foerst, and U. Kulozik. 2007. Damage of cell envelope of *Lactobacillus helveticus* during vacuum drying. *J. Appl. Microbiol.* 102:748–756.
- Takeda, N., M. Kato, and Y. Taniguchi. 1995a. Pressure- and thermally-induced reversible changes in the secondary structure of ribonuclease A studied by FT-IR spectroscopy. *Biochemistry* 34:5980–5987.
- Takeda, N., M. Kato, and Y. Taniguchi. 1995b. Pressure-induced secondary structure changes of ribonuclease A and ribonuclease S studied by FTIR spectroscopy. *Biospectroscopy* 1:207–216.
- Vermeirssen, V., J. Van Camp, K. Decroos, L. Van Wijmelbeke, and W. Verstraete. 2003. The impact of fermentation and in vitro digestion on the formation of angiotensin-I-converting enzyme inhibitory activity from pea and whey protein. *J. Dairy Sci.* 86:429–438.
- Winson, M. K., R. Goodacre, É. M. Timmins, A. Jones, B. K. Alsberg, A. M. Woodward, J. J. Rowland, and D. B. Kell. 1997. Diffuse reflectance absorbance spectroscopy taking in chemometrics (DRAS-TIC). A hyperspectral FT-IR-based approach to rapid screening for metabolite overproduction. *Anal. Chim. Acta* 348:273–282.
- Zeroual, W., C. Choisy, S. M. Doglia, H. Bobichon, J.-F. Angiboust, and M. Manfait. 1994. Monitoring of bacterial growth and structural analysis as probed by FT-IR spectroscopy. *Biochim. Biophys. Acta* 1222:171–178.