CONCLUSION

The present study deals with the isolation and thermotolerance study of *C. sakazakii* from milk and milk products of Agra city. Our results confirm the occurrence of *C. sakazakii* in cow raw milk, goat raw milk and buffalo raw milk and milk products. “Raw milk and milk products may be a source of *C. sakazakii* infections in infants, neonates and immunocompromised individuals” (Iversen and Forsythe, 2003) *sakazakii* contamination in raw milk may originate from the farm environment. Thus hygienic control measures and manufacture and consumer education strategies need to be established for the control of *C. sakazakii* infections. The results of thermotolerance study proved that *C. sakazakii* isolates and MTCC-2958 did not survive the pasteurization temperature and contamination of milk and milk products occurs during post-pasteurization or through the use of raw materials in minimum processed foods.

Proteomic analysis of whole cell protein by 1-D and 2-D gel electrophoresis and mass spectroscopy confirmed that *C. sakazakii* exhibit physiological differences between planktonic, agar-surface associated and biofilms cells. The biofilm-and planktonic proteomes were compared using 2-D gel electrophoresis. Nine proteins showed increased expression in biofilm cells. They were characterized by MALDI-TOF/TOF mass spectrometry. Proteins identified were classified as cell envelope – omp A and omp X, energy generation – enolase and triosephosphate isomerase, amino acid biosynthesis – shikimate kinase and membrane function - cystein-binding periplasmic protein.

Gaining knowledge about the biofilm proteome of *C. sakazakii* can help to design strategies that cause biofilm degradation, and thereby can subsequently help to prevent the contamination of food by biofilm formation on food contact surfaces by the above bacteria.

“In the present work, the phenotypic expression of planktonic, agar-surface associated and biofilm cells were also studied by FTIR and Raman spectroscopy” (Sharma and Prakash, 2014). “FTIR spectra of the agar-surface associated and biofilm cells exhibited higher intensity in the absorption bands assigned to polysaccharides, amide I, amide II and vibrational mode of ester” (Sharma and Prakash, 2014). “Raman
spectra of agar-surface associated and biofilm cells showed higher intensity in the absorption band assigned to tyrosine, amide III, carbohydrates, carotenoids, DNA and lipids” (Sharma and Prakash, 2014). FTIR and Raman spectroscopy were found to be relatively fast, simple, less expensive, non destructive and sensitive techniques that required a very small amount of sample. Spectra obtained from the two techniques provided information about the whole bacterial cell composition. The study using these techniques demonstrated that *C. sakazakii* that are agar-surface associated and the biofilm grown cells are chemically different from the planktonic cells. Nevertheless, further studies are needed to characterize and elucidate the biofilm cells of *C. sakazakii* grown in actual food environment conditions.

The study also focused on the susceptibility of *C. sakazakii* to antibiotics, plant products, and lactic acid bacteria. Minimum bactericidal concentration assays provided a good approach to select the antibiotics that are efficient in killing the bacteria growing as biofilms. Apart from antibiotics, extracts of plant parts and probiotic strains can be effective in controlling *C. sakazakii* infections, especially the drug-resistant strains. Therefore, the consumption of a diet supplemented with plant parts and probiotics under the present study may be used to prevent *C. sakazakii* infections. However, identification of specific molecules of plant extracts and lactic acid bacteria responsible for antimicrobial activity against *C. sakazakii* infection needs further investigation.