SUMMARY AND CONCLUSIONS

The field of probiosis has emerged as a new science with applications in human and animal use. The inherent resistance of bacterial spores to environmental stress is an attractive attribute to be used as probiotics. Bacterial spores are by far the most stable forms; which can resist harsh conditions of gastrointestinal tract. Spore formers especially Bacillus spp remain metabolically inactive during unfavourable conditions and during the onset of favourable conditions; spores will start to grow vegetatively. The collection of wild sporeformers of natural origin may likely provide a useful source for searching potential probiotics.

Eleven different isolates were selected from the fifty isolates, obtained from the natural sources, such as soil, dry spices, milk, intestine of pig and chicken, on the basis of primary probiotic screening criteria’s such as non haemolysis and non lecithinase activity. Spores and vegetative cells of the selected eleven isolates were tested for tolerance to acid and bile. From the results it was found that spores would render better results than vegetative forms. Based on acid and bile tolerance, eight spore formers were further selected and their resistance in artificial gastric and intestinal fluid were analyzed. The selected eight spore formers were studied for their antimicrobial activity with focus on their inhibitory action towards the enteric pathogens, such as Salmonella typhi, Salmonella paratyphi A, and Vibrio cholerae. Based on the results of antagonistic properties five isolates named MBTU PBBM1, S-7, CD-4, CD-1 and CI-2 were selected out from the eight isolates. Antagonistic principles such as siderophores and BLIS activity were studied for all these five isolates. Results revealed that none of these isolates produced siderophores. Antimicrobial substances produced
by the isolates in their cell free supernatant varied with respect to the action of proteolytic enzymes, Trypsin, Pepsin, Proteinase K and α-amylase. All the isolates were found to produce thermo labile antimicrobial compound except the isolate S-7. In this study, among the selected five isolates MBTU PBBM1, from milk showed remarkable probiotic characteristics and antagonism towards enteric pathogens *Salmonella typhi, Salmonella paratyphi* A and, *Vibrio cholera*. All the selected five isolates were further studied for adhesion properties.

Adhesion and cell surface properties are important attributes which help in the attachment of various substrata that explain the probiotic nature of the microorganism. Adhesion and cell surface properties of the selected five wild species of spore formers were compared with that of the enteric pathogens *Salmonella typhi, Salmonella paratyphi* A and *Vibrio cholera*. The present study revealed that the vegetative cells of the isolates were non hydrophobic and spores were found to be hydrophobic. Compared to the pathogens, spores were found to be more adhesive on intestinal mucous than vegetative cells. Both spores and vegetative cells were autoaggregating but autoaggregation of vegetative cells was found to be more than that of spores. Vegetative cells of isolates coaggregated the three pathogens while spores remained to be noncoaggregating. All five isolates and pathogens produced biofilm. In the case of enteric pathogens *Vibrio cholerae* registered a higher adhesion and cell surface properties than *Salmonella typhi, Salmonella paratyphi* A. Compared to enteric pathogens, MBTU PBBM1 possessed an enhanced adhesion and cell surface properties than other four isolates. Based on the *in vitro* studies the best performing probiotic isolate MBTUPBBM1 was selected for identification and further characterization.
Biochemical and molecular identification studies revealed that MBTU PBBM1 showed more similarity towards *Bacillus subtilis* with the NCBI gene accession number JN873913. Growth curve of MBTU PBBM1 had a short lag phase and short doubling time which are said to be the probiotic features to outcompete pathogens. There was no marked decline phase in the growth curve due to the presence of spores. Optimal growth temperature of this strain was around 35°-40°C. MBTU PBBM1 possessed a plasmid of ~12 kb. The strain was studied for susceptibility towards antibiotics and found that it was sensitive to all the tested antibiotics except Methicillin. Plasmid curing experiment was performed in order to check the role of plasmid in antagonistic activity. However, it was inferred that the plasmid had no role on the antagonistic property of MBTU PBBM1. Studies also revealed that Methicillin resistance was plasmid mediated. Cured strain of MBTU PBBM1 was Methicillin sensitive which add probiotic value of MBTU PBBM1 to be used as probiotic because this may limit the transfer of many antibiotic resistance genes. Health promoting effects of MBTU PBBM1 was studied by determining the ability to deconjugate bile salt and to assimilate cholesterol. Results revealed that MBTU PBBM1 deconjugate sodium taurocholate and remove cholesterol from growth media.

Probiotic properties of a particular strain are only full filled with *in vivo* studies. *In vivo* study investigated the *in vivo* effects of 30 days consumption of MBTUPBBM1 on general health, humoral and cell mediated immune response as well as the potential infectivity and pathogenicity. In the study, spores of MBTU PBBM1 was given in two doses as a feed supplement in the diet of Balb/c mice and the effect on immunomodulation was studied with focus on the animal’s humoral and
cell mediated immune responses. Dose dependent studies suggested that, 1x10^8 spores as the suitable dose to increase both humoral and cell mediated immunity. Persistence studies revealed that MBTU PBBM1 can effectively colonize the gastrointestinal tract. No apparent significance was observed in haematology and total behaviour of both treated and non treated group. Humoral immune response studies revealed that MBTU PBBM1 spores induced an increase of serum Ig A, Ig G and antibody responsiveness to SRBC in mice. Macrophage activation was studied by NBT assay. Results revealed that spore treatment enhanced nitroblue tetrazolium reduction which further helped in oxygen dependent bactericidal activity. Delayed type hypersensitivity (DTH) studies indicated that spore treated animals showed increased DTH than control groups. MBTU PBBM1 spores administration in mice, stimulated the spleen cell proliferation in response to Concanavalin A. No incidence of bacterial translocation was observed in spore treated group. Viable counts in ileac section again confirm the colonization capability of MBTU PBBM1 in GIT tract. Histological examination showed that feeding mice with MBTU PBBM1 spores caused no signs of inflammation, degeneration or necrosis of the intestinal mucosa.

Immunomodulatory studies revealed that MBTU PBBM1 can enhance both humoral and cell mediated immunity. So the preventive and curative role of MBTU PBBM1 strain in immunosuppression was studied with focus on the animal’s haematology, immune responses and histological alterations in small intestine. The results revealed that MBTU PBBM1 treatment can prevent immunosuppression and has a capacity to increase immune response in such conditions. Histopathological results also supports the preventive and curative capabilities of MBTU PBBM1 in
Summary and Conclusions

CTX induced immunosuppression. This enhances the therapeutic benefit of this probiotic for patients at the risk of immunosuppression especially in cancer patients.

The potential probiotic strain MBTU PBBM1 from milk has strong probiotic properties such as tolerance to acid and bile, non haemolytic, lecithinase negative, resistance to artificial gastric and intestinal fluid and antagonism to enteric pathogens such as *Salmonella typhi*, *Salmonella paratyphi A*, and *Vibrio cholera*. MBTU PBBM1 possessed enhanced adhesion to intestinal mucous than enteric pathogens revealing its ability for gastrointestinal adhesion. Isolate also shows superior adhesion and cell surface properties than enteric pathogens which help in exclusion of enteric pathogens. In addition to this, MBTU PBBM1 possessed health promoting effects such as bile salt deconjugation and cholesterol assimilation. Immunomodulatory studies also proved that the strain has a significant role in improving both humoral and cell mediated immunity. CTX induced immunosuppressive studies showed that MBTU PBBM1 could potentially prevent and reverse immunosuppressive effects of cyclophosphamide in the various immune parameters measured. All the studies revealed that this probiotic strain has promising probiotic potential in managing nutritional status, disease outbreaks and regarded as GRAS(generally recognized as safe) status. The above potentials of MBTU PBBM1 provide a strong information in considering the efficacy of this probiotic strain as nutraceutical and in probiotic therapy.