

## **Chapter 8**

# **SUMMARY AND FUTURE PROSPECTS**

## Summary

- The AMD17 isolated from goat milk showing potential probiotic attributes was identified as *Lactococcus lactis*.
- The isolate significantly reduced the adherence of foodborne pathogen *Listeria monocytogenes* AMDK2 ( $47.46 \pm 0.17$  %) to Caco-2 cells.
- Honey was used as an adjuvant of *L. lactis* AMD17 for preparation of dahi (curd) and was found to support its survivability during storage ( $P < 0.05$ ).
- Sensory evaluation studies revealed that dahi prepared with *L. lactis* AMD17 and the addition of 3 % honey exhibited the highest score in taste and color.
- AMD6 isolated from Doi was identified as *Lactobacillus plantarum* AMD6, displayed good tolerance to gastrointestinal stress conditions and showed potential attributes for cholesterol assimilation.
- The recombinant AMD6 BSH-His showed a relatively high preference for glycine-conjugated bile salts.
- The HFD+AMD6 animal showed significant reduction of serum TC, TG, and LDL-C levels to 65.45, 123.30, and 16.89 mg/dl, respectively, after 6 weeks treatment. Therefore, AMD6 have the potential to be explored as probiotics in the management of cardiovascular diseases.
- The AMS1 isolated from Churpi showed gastrointestinal transit tolerance, cell surface hydrophobicity, cell aggregation and antimicrobial activity against food borne pathogen indicator strains. It also showed a remarkable cellulolytic activity as confirmed by the SEM studies.
- The isolate was also able to degrade filter paper within 96 hours of incubation.
- Cellulase encoding gene from potential probiotic *Bacillus amyloliquefacies* AMS1 was identified and found to be of approximately 1500 base pairs.

- Docking result showed that CMC binds to cellulase AMS1 with a binding energy of  $-7.97648 \text{ kJ mol}^{-1}$  and showed the presence of 12 putative residues of cellulase AMS1 that contact with CMC through hydrophobic interaction (Asn134, Lys33, His131, Gln297, Lys296 and Trp69) and hydrogen bonding with Ala98, Asp99, Thr97, Ala36, His65.
- The purified recombinant cellulase protein was characterized and shows maximum activity at pH 5.0 (0.41 U/ml). It also showed maximum activity within a broad range of temperature (10 °C - 90 °C) with an optimum at 50 °C (0.43 U/ml).
- The probiotic attributes of *Bacillus subtilis* AMS6 isolated from fermented soybean (Churpi) exhibited tolerance to low pH (pH 2.0) and bile salt (0.3 %), capability to autoaggregate and coaggregate.
- AMS6 also showed highest antibacterial activity against the pathogenic indicator strain *Salmonella enterica typhimurium* (MTCC 1252)
- Further studies revealed *B. subtilis* AMS6 showed cellulolytic activity ( $0.54 \pm 0.05$  filter paper units  $\text{mL}^{-1}$ ) at 37 °C.
- The isolate was found to hydrolyze carboxymethyl cellulose, filter paper and maize (*Zea mays*) straw. The maize straw digestion was confirmed by scanning electron microscopy studies. The isolate was able to degrade filter paper within 96 hours of incubation.
- A full length cellulase gene from AMS6 consisting of 1499 nucleotides was identified and showed that the enzyme belongs to glycosyl hydrolase 5 family.
- The study suggests potential probiotic *Bacillus subtilis* AMS6 as a promising candidate envisaging its application as an animal feed additive for enhanced fiber digestion and gut health of animal.

### **Future prospects**

1. Understanding molecular mechanism of cholesterol assimilation properties of *Lactobacillus plantarum* AMD6.
2. Preparation of functional food by incorporating *Lactobacillus plantarum* AMD6.
3. Purification of cellulase enzyme and assessing its potential application in feed industries.