SUMMARY
The present research work entitled “Characterization of traditional fermentation processes used for the production of some alcoholic beverages (Chhang, Sura and Jau Chhang) in Himachal Pradesh” was carried out to characterize these traditional fermented products and also the indigenous fermentation processes developed by the tribal and rural folks of Lahaul & Spiti and Kullu districts of Himachal Pradesh. The major findings reported in this thesis are summarized below:

1. Status of the traditional fermented beverages of Himachal Pradesh

Various cereal, fruit and Jaggery based fermented alcoholic beverages such as chhang, jau chhang, sura, angoori, bhemi and daru etc. are very popular among the tribal and rural people of Lahaul & Spiti, Kinnaur and Kullu districts of Himachal Pradesh. These beverages form a very important part of the socio-cultural life of the people in these districts. During religious ceremonies, local festivals and marriages it is customary to consume these cereal and fruit based indigenous beverages. ‘Phab’ the traditional inoculum is used in the preparation of some of these beverages. In others locally made additives like dheli (made of 36 herbs and roasted barley flour) is used.

2. Microbiological studies of chhang, jau chhang and sura fermentation

The identification of the predominant microflora in the traditional fermented beverages by 16S rDNA (bacteria) and 26S rDNA (yeasts) revealed that Saccharomyces cerevisiae, Saccharomyces fibuligera, Lactobacillus plantarum, Pediococcus pentosaceus and Enterococcus pentosaceus were the main fermenting organisms common to chhang, jau chhang and sura fermentation. Besides these other bacterial species like Bacillus amyloliquefaciens and Serratia sp. were also associated with chhang and jau chhang fermentation. In addition Enterococcus lactis and Lactobacillus casei were the other lactic acid bacterial species associated with these fermentations. Among other yeast species identified were Pichia kudriavzevii from sura and Candida tropicalis from jau chhang. Increase in microbial counts of both
yeasts and lactic acid bacteria were observed in chhang and jau chhang fermentation initially for 3 days. Initial increase in counts was followed by a gradual decrease. In sura, the counts of both LAB and yeasts decreased with progress in fermentation. With increase in microbial load during fermentation, pH decreased from initial 6.2 to 3.4 at the end of 7th day of chhang fermentation. Also it decreased from 6.2 to 4.4 for jau chhang and from 5.0 to 3.6 at the end of 10th day of spontaneous fermentation initially for sura and finally it decreased further to 2.9 in 20th day ferment. An increase in total acidity was observed in all the three fermented beverages with the decrease in pH.

3. **Biochemical studies of chang, jau chhang and sura fermentation**

During chhang fermentation, total proteins increased from initial 7.2% to 13% (w/w), total carbohydrates decreased from 76.8 to 17.6% (w/w), starch from 56% to 9.5% (w/w) on dry weight basis. However, reducing sugar level of the rice ferment increased from initial 2.3% to 11% (w/w) at the end of 7th day of chhang fermentation. The amylase activity increased from initial 0.22 U/g (dry weight basis) to 0.98 U/g after 3 days of fermentation but later on declined. Proteolytic activity also showed the same trend with initial increase from 0.01 U/g to 0.04 U/g in 2nd day rice ferment and then decreased finally to 0.01U. Fermentation of cooked rice significantly enhanced the B vitamin levels especially thiamine (B₁), pyridoxine (B₆) and folic acid (B₉). Amino acid analysis also revealed enhanced levels after fermentation. Some of the essential amino acids (EAA) like tryptophan, alanine increased quite significantly post fermentation in the rice chhang. Though some amino acids showed a decrease also.

In jau chhang fermentation (8 days), protein content increased from 4.4% to 20.2% (w/w on dry weight basis), total carbohydrates reduced from 63% to 12% (w/w) with increase in days of fermentation. Also starch content in jau chhang ferment decreased from 27.3% to 15.2% (w/w) by the end of 8th day. Jau chhang has reducing sugar content of 1.17 % (w/w). Amylase and protease activity increased at initial stages and later on declined. Fermentation significantly enhanced the B vitamin levels especially riboflavin (B₂), niacin (B₃) and pyridoxine (B₆) and essential amino acids like alanine, threonine and leucine (EAA) showed a substantial increase in the final jau chhang ferment.
Sura, millet based fermented beverage also showed an increase in protein content from initial 8% (w/w) to 12.8% (w/w) after 20 days of fermentation. Total carbohydrates decreased from 60% (w/w) to 11% (w/w), starch from 55.3% (w/w) to 16.32% (w/w). Levels of reducing sugars, however, increased with progress in fermentation and were detected at a level of 3% (w/w) on dry weight basis in the 20th day ferment. There was an increase in amylase activity from initial 0.04 U/g (on dry weight basis) to 0.23 U/g after 15 days of fermentation but later on a decrease was observed. Proteolytic activity of 0.07 U/g (on dry weight basis) was observed in the 20th day ferment. B vitamins are significantly increased post fermentation especially riboflavin (B2), niacin (B3), pyridoxine (B6) and cyanocobalmin (B12). Amino acid content increased during fermentation. There was a significant increase in level of valine and leucine in 20th day Sura ferment. Some of the amino acids like aspartic acid and proline were the additional amino acids detected in the ferment (sura).

4. **Volatile analysis of the fermented beverages**

Ethanol was the major volatiles detected in all the three cereal based alcoholic beverages. The alcohol content gradually increased with progress of fermentation. In rice chhang ethanol content increased to 4.6% (w/w) by 5th day but thereafter it declined. Ethanol content in both jau chhang and sura were 5% (w/w) after final fermentation. Acetic acid was the organic acid detected at a level of 1.8% (w/w) in chhang and 1.2% (w/w) in 10th day Jau chhang ferment. No acetic acid was detected in sura.

5. **Improvement of chhang using the standard starter cultures**

Best chhang in terms of sensory quality and saccharification was obtained using the co starters i.e. L. rhamnosus + L. casei + Streptococcus salivarius + Bacillus amyloliquefaciens. However the 'phab' inoculated traditional chhang was still the best in terms of flavor and aroma.

6. **Probiotic/functional aspects of traditionally fermented beverages**

Functional aspects of sura were proved by analysis of dheli (concoction of 36 herbal extracts and roasted barley flour) the traditional additive used in sura preparation. Quercetin one of the flavonoids was detected at concentration of 1.87%
The methanolic extract of dheli showed an antioxidant activity of 26%. Thus proving the efficacy of sura as a potential functional beverage.

The probiotic properties of the lactic acid bacterial isolates were evaluated. All the LAB isolates were studied for their antimicrobial activity against five indicator bacterial strains i.e. Shigella dysenteriae, Staphylococcus aureus, E. coli, Listeria sp. and Bacillus cereus using well diffusion assay. Only few LAB isolates J14 (L. plantarum), J22 (L. plantarum) inhibited Bacillus cereus. Isolate J23 inhibited Listeria sp. All LAB isolates exhibited good acid tolerance on incubation for 3h at pH 2, pH 3 and pH 7. Isolates J15 (L. plantarum), J20 (Lactobacillus sp.), J21 (Pediococcus pentosaceus) and J24 (L. casei) were the best acid tolerant organisms.

All the LAB isolates exhibited very good tolerance of bile at different concentrations of (0.5%, 1% and 2%) on incubation for 24 h. The LAB isolates exhibited exopolysaccharide production, showed no haemolytic and proteolytic activity.

The results of this study revealed that fermented rice (chhang), barley (jau chhang) and ragi (sura) are nutritionally more beneficial than the unfermented cereals. All the three fermented cereal based alcoholic beverages contained a variety of yeasts and lactic acid bacteria that contribute towards the flavor and preservation of these beverages. In addition to being alcoholic in nature these beverages have better nutritional value with significant amounts of B-vitamins and essential amino acids as a result of fermentation. Also the use of mixed starters “phab” in these traditional fermentations is an innovative technology that uses the native skills of tribal people in maintaining the essential consortia of microorganisms. Cereal based fermented beverages are thus a suitable alternative to milk–based carriers for probiotic bacteria as these have high counts of viable lactic acid bacteria and may serve as a blueprint for development of cereal-based probiotic functional beverages. Thus it is concluded that these traditionally fermented cereal beverages have potential nutritional vis-à-vis probiotic and functional benefits to the consumers.