SUMMARY AND CONCLUSION

The aim of present study was to explore the diversity of psychrotolerant bacterial isolates from the Himalayan regions, secreting $\beta$-galactosidase enzyme. To achieve this aim, we isolated 14 bacterial isolates from soil samples of Kafnu valley and Sangla valley, which remains snow covered throughout the year. Out of the 14 isolates, only 5 isolates (A2, A3, A5-1, A5-2 and B8) produced $\beta$-galactosidase enzyme extracellularly and constitutively. The $\beta$-galactosidase was also found inducible in nature. Among 5 isolates, A5-2 and B8 showed the highest $\beta$-galactosidase activity in cell free spent medium. The biochemical characterization and 16s rDNA sequencing revealed that both isolates belong to Enterobacteriaceae family and has been identified as *Serratia quinivorans* strain A5-2 and *Serratia quinivorans* strain B8 by 16s rDNA nucleotide sequencing. Both of the nucleotide sequences have been submitted at the NCBI gene bank database with accession number KJ 176660 and KJ 176661 respectively.

Till date, not much is known about *Serratia quinivorans* as a psychrotolerant. Moreover, the $\beta$-galactosidase enzyme in *Serratia quinivorans* has been characterized for the first time. Surprisingly, there is no report about extracellular $\beta$-galactosidase from psychrophilic/psychrotolerant bacteria till date.

Biochemical characterization of $\beta$-galactosidase enzyme showed that the enzyme is constitutive as well as inducible. There was 2 folds and 3 folds induction in the $\beta$-galactosidase activity in A5-2 and B8 isolates respectively, in nutrient medium supplemented 1% lactose. The enzyme was optimally active at pH 7 in both the isolates. Surprisingly, enzyme was found thermostable in nature, as it showed its optimal activity at 50˚ C in A5-2 isolate and 60˚ C in B8 isolate. The enzyme retained 100% active when tored at 4˚ C. it was very surprising that highest $\beta$-galactosidase production was observed during death phase in both the isolates. The maximum activity observed was 9000 U/mg in A5-2 isolate and 23000 U/mg in B8 isolate in nutrient medium supplemented with 1% lactose. The major milk sugars such as Ca$^{2+}$ and Na$^+$ did not affect the enzyme activity. The $\beta$-galactosidase activity was induced by Fe$^{2+}$ and Ba$^{2+}$ ions. Both A5-2 and B8 isolates showed growth in medium supplemented with heavy metals like Ba$^{2+}$. In contrast, presence of sugars (lactose, galactose and glucose) in enzyme assay did not inhibit the activity. The $\beta$-galactosidase of both the isolates showed the efficient bonding with ONPG and
the substrate affinity ($k_m$) was observed 0.07 mM in A5-2 isolate at 50° C and 0.2 mM in B8 isolate at 60° C. The SDS-PAGE and zymogram revealed the size of β-galactosidase ~100 KDa for *S. quinivorans* A5-2 and 70 KDa for *S. quinivorans* B8.

Thus, it is concluded that β-galactosidase from psychrotolerant *S. quinivorans* A5-2 and *S. quinivorans* B8 could be further purified and utilized for the treatment of lactose product, to reduce the lactose. Moreover, both the isolates grow below 30° C and hence could be utilized for bioremediation of milk based effluents at colder region.