CONCLUSIONS
The following conclusions are drawn from the results of the present investigation.

1. Bacterial isolate NFB-5 has been found the most potent aspartase producer and its molecular characterization by 16S rDNA sequencing as well as phylogenetic analysis confirmed it to be *Aeromonas media*.

2. Primers designed from conserved sequences KMGRTQ/HLQDA and MPA/GKVNP successfully amplified the central domain of aspartase gene (*aspA*). Further, primer designed from central domain and *E. coli* aspartase amplified the N- and C-terminal sequence of aspartase (*aspA*) gene which nullified the need of genome walking or genomic library construction.

3. Key amino acid residues (Arg 29, Lys 327, Arg 15 and Asp 10) which are reported to involve in enzyme-substrate interaction and catalytic function were found conserved in *A. media* NFB-5 aspartase. The amino acid sequence also showed homology with class II fumarase. The Ile/Ile + Val + Leu ratio of *Aeromonas media* aspartase was found equivalent to *Pseudomonas fluorescens* aspartase and lesser than thermophilic aspartase from *Bacillus* sp. YM55-1 and *Cytophaga* KUC-1.

4. Aspartase gene (*aspA*) was successfully cloned in pET21 DNA vector and transformed in *E. coli* for its expression. Optimization of expression conditions and media supplements at shake-flask level showed almost 2-fold increase in aspartase production. IPTG concentration, induction time and salts concentrations were found effective for aspartase expression, while biotin remained futile.

5. Response surface optimization of aspartase production at laboratory scale bioreactor resulted in more than 2-fold increase as compared to shake-flask level.
Conclusions

Interaction among the aeration, agitation and induction time was found to regulate the aspartase production. Experimental run under optimal conditions validated the predicted responses.

6. The recombinant aspartase was purified to homogeneity by single-step purification with a 32-fold increase in specific activity. Purified recombinant aspartase showed moderate pH stability and lower thermostability as compared to aspartase from Cytophaga sp. KUC-1 and Bacillus sp. YM55-1. The difference in thermostability was also supported by difference in their Ile/Ile + Val + Leu ratio. Metal ions requirement was found similar to E. coli aspartase.

7. The data on half-life, D-value and Z-value showed that enzyme stability is lower at higher temperature. D-value and half-life were found to decrease with increasing temperature. Similar response was obtained for ΔH° which suggested that lesser energy is required for denaturation of enzyme at higher temperature. The negative value of change in entropy showed the significant processes of aggregation at elevated temperatures.

8. Triton X-100 was found to be the most efficient permeabilization agent for recombinant E. coli cells. Immobilization of permeabilized cells followed by homogenization resulted in higher production of aspartic acid. Homogenized immobilized cells under agitation yield more than 3-fold L-aspartic acid as compared to permeabilized free cells.

The investigations carried out on Aeromonas media NFB-5 and recombinant aspartase would provide useful guidelines to execute further research on bacterial aspartases for L-aspartic acid production.