6. Summary

Several bioinformatics tools, softwares and databases were used to study the multiple genes coding for ß-lactamases (BLA), ß-lactamase domain containing proteins (BDP) and penicillin binding proteins (PBP) that contributed to high level of resistance to ß-lactam antibiotics. The conserved domain database (CDD) analysis of BDPs predicts them to be member of metallo-ß-lactamase-superfamily. Further, class C specific multi-domain AmpC (beta-lactamase C) was found in the three ß-lactamases. The β-lactam resistance-determinant motifs, HXHXD, KXG, SXXK, SXN and YXN are present in the BLAs, BDPs and PBPs of *Z. mobilis*. The predicted theoretical isoelectric point and aliphatic index values suggested their stability at wide-range of pH and temperatures. The phylogenetic examination of the amino acid sequences of all ß-lactam resistance-determinants has indicated to share a common ancestor with the reported ß-lactam resistance in bacteria. One of the penicillin binding proteins, PBP2 was predicted to have interactions with the rod-shape-determining proteins (YP_162095 and YP_162091). The three dimensional structures of the ß-lactam resistance determinants were derived through homology modelling and subsequent docking studies has specifically indicated KXG motifs to interact with penicillin. *Z. mobilis* ZM4 was found to be resistant to penicillins, cephalosporins, carbapenems, narrow, broad and extended spectrum ß-lactam antibiotics. The substrate specificity of the ß-lactam resistance-determinants are confirmed through disk diffusion assay, SDS-PAGE and zymogram analysis. The hypothesis proposed through the computational analysis was experimentally confirmed by atomic force microscopic analysis of cell structures before and after treatment with penicillin. Semi-quantitative PCR analysis has indicated the induction and overexpression of all BLAs and one BDP upon exposure to penicillin. This study provided insights into the

prevalence of ß-lactam resistance-determinants in a non-pathogenic bacterium that could have implications in natural and laboratory settings as such this bacterium is routinely used in many research laboratories as an industrial model organism. The present study is the most comprehensive one on resistance genes so far in any non-pathogenic bacteria.

Preliminary sequence analysis revealed the presence of gene products namely cellulose synthase catalytic subunit (ZMO1083), cellulose synthase regulator protein (ZMO1084), cellulose synthase operon C domain-containing protein (ZMO1085) necessary for cellulose production. Conserved domain database analysis and motif search revealed the presence of characteristic domains and motifs necessary for cellulose production. Moreover, the functional relationship between genes in cellulose synthase operon was found to significant in terms of neighbourhood, gene fusion and co-occurrence data. The synthesis of cellulose by *Z. mobilis* was confirmed by Congo Red, Calcofluor and Updegraff cellulose assays and visualized by fluorescence and atomic force microscopic techniques. Transcript analysis of cellulose synthase operon genes indicated that transcripts of the *bcsA, bcsB* and *bcsC* and the intervening regions between the *bcsA-bcsB* and *bcsB-bcsC* were at higher level when compared to the endogenous control *adhB*, alcohol dehydrogenase B. Transcript of the *bcsC* was at lower level when compared to the endogenous control *adhB*, alcohol dehydrogenase B.

Preliminary sequence analysis revealed the role of beta-galactosidase as hyrolase activity of O-glycosyl compounds. Conserved domain database analysis and motif search revealed the presence of characteristic domains and motifs necessary for

beta-galactosidase activity. Further, three dimensional structure prediction and further docking of beta-galactosidase revealed greater level of interaction with the lactose molecule. The absolute copy concentration of beta-galactosidase was determined to be 2 from the standard curve using the C_t value. The transcript of beta-galactosidase gene was found to be 4-fold lower than that of *adhB* transcript. Beta-galactosidase activity was not detected in extracellular fraction of *Z. mobilis* B-14023. In intracellular fraction of *Z. mobilis* B-14023 clone, beta-galactosidase activity was found to be detectable by qualitatively. The optimal pH and temperature for the purified beta-galactosidase was determined to be 8.0 and 30 ºC. The maximum specificity of the beta-galactosidase was observed on the substrates such as ONPG and PNPG.