Richa Bajpai (2009). ***Molecular and functional analysis of integrase gene of bacteriphase PIS 136.*** Ph.D. Thesis. CSIR-IMTECH, Chandigarh/ Jawaharlal Nehru University, New Delhi: India.
Supervisor: Dr. Pushpa Agrewal **Acc. No. : Th-212**

SUMMARY OF THE THESIS

So far our study of phage PIS 136 has shown that it has a wide host range with a relatively large double stranded DNA genome. Amino acids sequence analysis of PISint protein using alignment tools indicated that PISint, which is a subject of the present study, is likely to be a site specific recombinase, however none of the results suggested that PISint could be a site specific recombinase. Thus, PISint though has been referred as an 'integrase' in this report, functionally does not fall in to the category of a classical 'integrase' protein. PISint did bind to the cruciform structure located upstream to the *PISint* gene which in general ought to

be the *attP* site, at least theoretically; however experimental data did not confirm this assumption. PISint bound very efficiently with the cruciform structure but failed to bind to DNA fragment which did not have any secondary structure. The result clearly indicated its preference for a certain DNA topology/ possible structure selectivity. Yet another property of PISint was that it readily formed co-integrates both *in vivo* and *in vitro*. Surprisingly, every recombination site contained 'CA' dinucleotide, which led to the assumption that PISint might be a transposase. PISint protein could not be purified in native form thus was purified from inclusion bodies under denaturing condition (8M urea) and refolded, where urea concentration could be reduced to 2M. Protein was stable at this concentration of urea and also seems to have no effect on the biochemical property of the protein. PISint in 2M urea bound to DNA and also showed structure selective endonuclease activity similar to that of T7 endonuclease I. PISint showed functional similarity to T7 endonuclease I therefore it was essential to know the recognition and cleavage point of PISint in a supercoiled DNA harboring the cruciform structure to which PISint had shown binding. Sequence analysis of the linear form of the supercoiled plasmid generated after PISint treatment showed that PISint indeed cleaves the stem region of the cruciform structure at the position indicated by down arrow ACAATGTGAGA↓GG in both the strand of DNA.

So far the results showed that PISint is a recombinase, thus it was important to know the nucleophilic amino acid of the protein. Several mutations were generated which were based upon the sequence information *e.g.* Tyr^{351}: a possible nucleophile if PISint was a site specific recombinase; Ser^{9}, if it was a resolvase, Asp^{22}, Asp^{39} and Glu^{86}, assuming that PISint was a transposase and Arg^{201}, Arg^{319}, Tyr^{316} were mutated beacsue they were conserved in all tyrosine recombinases while His^{342} was identified as a possible nucleoplile after molecular modeling of PISint-DNA complex. However, proteins of Asp^{39} and Glu^{86}, Arg^{201} and Arg^{319} mutation did not express even though clones were perfectly fine. Therefore, we assume that these mutations would have caused major conformational change in the protein, as discussed earlier. Remaining mutated proteins were functionally analysed. None of these mutations affected the protein both physically (biophysically) which was evident from their extent of refolding as well as solubility. Mutation affected PISint functionally to some extent but the basic property of the mutant proteins were similar to that of PISintWT, as all of them bound to

the cruciform structure and showed endonuclease activity. A Tyr^{351} and Ser^{9} double mutant protein was hyperactive instead of losing its activity which indicated that neither of the two residue was a nucleophile. Nonetheless, few points were clear (a) PISint is a DNA binding protein, (b) it binds to a secondary structure of DNA rather than a linear DNA, (c) it forms co-integrates where every recombination point has 'CA' di-ncleotide and (d) the protein functions as a structure selective endonuclease. From all the data described in here, it appears that PISint is closer to transposases, which use water as nucleophile rather than a particular amino acid thus avoids any site specificity. This property offers lot of fluidity to PISint and helps it to infect several genera at multiple sites successfully. Finally, it is tempting to think that PISint is actually a chimera and may be a recently evolved phage, which still carries sequences similar to a site specific recombinase but has moved further to make its own identity.