7 Summary

The phylogenic characterisation of a new pathogenicity island (LEE) of strain RW1374 (O103:H2) and other STEC strains

The locus of enterocyte effacement pathogenicity island (LEE PAI) is a major virulence feature in enteropathogenic bacteria. The origin and spread of that genomic island during the evolutionary history of enterobacteria is poorly understood, therefore one of the major issue of this report consists in unravelling parts of the LEE history.

In the present study, LEE insertion sites in various pathogens (*E. coli*, *C. rodentium*, *E. alvei*) unravelled by PCR analysis and hybridisation confirms that the island inserted at multiple occasions into various sites during the evolution of enteropathogenic and enterohemorrhagic *E. coli* lineages. The fact that different kind of bacteria, strains of various clonal clusters and pathotypes of different origin express the same intimin type but have different LEE location strongly supports this hypothesis. By the way, the various intimin types are based on a mosaic structure due to LEE internal recombination. However, the negative result of hybridisation with the intimin probe of a human AE positive DEC strain suggests that this might be due to a specific mobilisation process of the intimin gene which only results in the spread of the intimin gene instead of the entire LEE. Contrary to the current opinion it was shown by hybridisation that EPEC2 strains possess a LEE inserted in *pheV* locus. DEC clones 9 of EHEC2 displayed a LEE inserted in *pheU*. However, strains of these different clonal clusters have the same intimin type (β), while Dec clones 8 of EHEC2 displayed a LEE inserted in *pheV* but have intimin type θ. These results imply that two divergent LEE bearing elements might have been acquired either simultaneously by different cells from a common ancestral strain of EPEC2/EHEC2 lineages or separately by its progenitors. Human *E. coli* as well as bovine *E. coli* results confirmed a direct relationship between intimin type and LEE insertion sites. Most of the bovine strains possessed a LEE inserted in *pheU* and have a β intimin type. The same was true for one *C. rodentium* strain (DBS125). All *E. alvei* strains displayed a LEE inserted in *pheU*, too, but they have an α intimin type. The description of a possible new phylogenetic lineage of AE positive pathogens is based on an apparent correlation between LEE insertion site and intimin type. The investigation of a common LEE insertion site (*pheU* or *pheV*) of various bovine EHEC strains which all express a common intimin type (ε or ζ) indicates the evolution of further clonal lineages of *E. coli*.

PCR analysis of LEE insertion sites in *E. coli* strains revealed that most of the strains possessed a second genomic island in addition to the LEE PAI inserted in the corresponding *pheU* and *pheV* locus, respectively. Hence, we developed a model of integration of a genomic island in *pheV* and *pheU* gene. This scenario fits well into the model of evolution of PAIs presumed by Hacker and Kaper (51). A putative PAI precursor integrated presumably in one or few *E. coli* ancestors, evolved and disseminated in parallel with evolution of the contemporary phylogenetic lineages of *E. coli*. LEE was apparently acquired after the primary genomic island was integrated. Mutations and deletions on the junctions between PAI and transfer-RNA gene are often involved in PAIs stabilisation. An indication of such deletions at the right LEE flanking region deliver the results of hybridisation due to absent hybridisation signals.

A genomic element can be acquired by a bacterial cell in three different ways (conjugation, transformation, transduction), in theory. The LEE of the bovine EHEC strain RW1374 possesses features which are congruent with the acquisition of the LEE via conjugative transposition. The intimin (*eae*) gene of the LEE_{RW1374} was marked with an insertion plasmid to mobilize the LEE. It was not possible to mobilize the LEE in an accomplished conjugation experiment, however this does not rule out a formerly transfer of the LEE via conjugation.

Methods to mark both sides of the *pheV* inserted LEE PAI of RW1374 were used to isolate this island. For this purpose we used insertion plasmids, that carry recognition sites for the rare cutting restriction enzyme *Not*I. Nevertheless, only one side of the *pheV* located island was successfully marked via homolog recombination. pSG76-A was introduced on the left *pheV* flanking region, and pST76-A was introduced on the right *pheV* flanking region. Also cloning of the LEE PAI of RW1374 in a non-pathogenic *E. coli* K12 strain was not achieved. Application of these two methods to another *E. coli* (3030A-86) and another *C. rodentium* (DBS100) strain failed, too. However, the pathogenic properties of the partial marked strains RW1374::pSG76-A and RW1374::pST76-A as well as the intimin mutated strain RW1374::*eae*pST76-A were characterised in a cell culture assay (FAS test). It was shown that a partial marking of the LEE PAI flanking regions has no influence on attaching and effacing lesions typical for LEE containing pathogens. On the contrary, the intimin mutated strain failed to form AE lesions in HEp2 cell culture. This result indicated that intimin-mutated strains lost their ability to form AE lesions.