Plants and their Pathogens

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Formation of the type III secretion pilus of *Xanthomonas campestris* pv. *vesicatoria* depends on the action of a pilus assembly protein

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The Gram-negative plant pathogenic bacterium *Xanthomonas campestris* pv. *vesicatoria* utilizes a type III secretion (T3S) system to translocate bacterial effector proteins into eukaryotic host cells and thus to trigger processes in the host necessary for bacterial infection. (Fig. 1). The T3S system consists of a basal apparatus localized in the inner and outer bacterial membrane which is connected to a pilus (outer diameter 6 nm) that penetrates the plant cell wall during infection. Translocation of effector proteins is mediated by a special translocon which inserts into the plasma membrane of the host cell.

Effector protein transport depends on the early T3S substrate HrpB2 which is essential for the assembly of the extracellular pilus of the T3S system. To characterize the role of HrpB2 during T3S and pilus formation we performed a transposon mutagenesis approach which led to the insertion of pentapeptide-encoding sequences into *hrpB2.* Complementation studies revealed that the N-terminal region of HrpB2 tolerates insertions whereas pentapeptide insertions in the central (amino acids 60-74) and the C-terminal region (amino acids 93-130) resulted in a loss of protein function. The C-terminal region of HrpB2 contains a conserved amino acid motif that is present in HrpB2 homologs and predicted inner rod proteins from animal pathogenic bacteria. Mutant studies showed that this motif is required for the contribution of HrpB2 to T3S, pathogenicity and pilus formation and is also essential for T3S of HrpB2 itself, suggesting that HrpB2 promotes its own transport.

Immunogoldlabeling and fractionation studies revealed that HrpB2 is presumably not associated with the T3S pilus but localizes to the bacterial periplasm and the bacterial outer membrane (Fig. 2). For immunoldlabelling bacteria were grown for 6 hours on formvar-coated gold grids, shortly fixed with paraformaldehyde and thereafter treated with antibodies.

To characterize functional areas of HrpB2, derivatives of this protein with amino acid exchanges or lacking certain regions were created. Pilus formation of bacteria expressing the mutated HrpB2 was compared with pilus formation by the wildtype. The pilus formation was determined by negative staining. We could show that HrpB2 is essential for pilus formation and that the amino acids in position 123 to 128 are important for protein function.

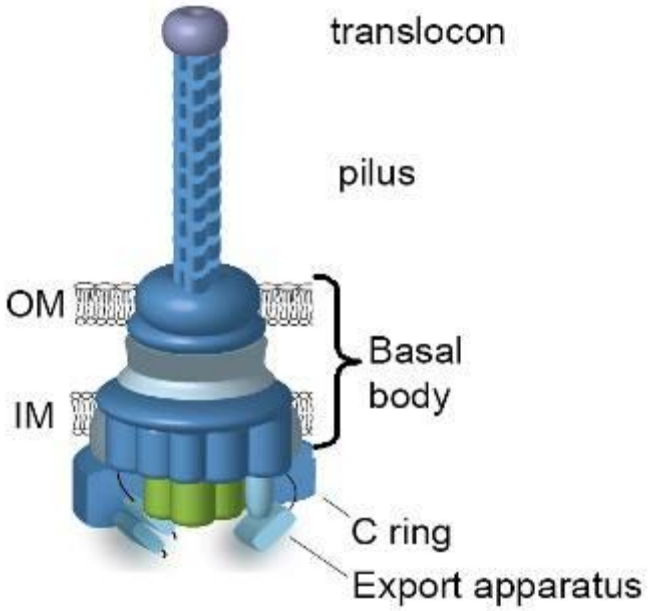

Figure 1. Schematic drawing of the T3S apparatus of *Xanthomonas campestris* pv. *vesicatoria* consisting of cytoplasmic C ring and export apparatus, a basal body located in the membranes and the periplasm, a pilus and a trranslocon. Adapted from Büttner Microbiol. Mol. Biol. Rev. (2012) 76(2) 262-310.

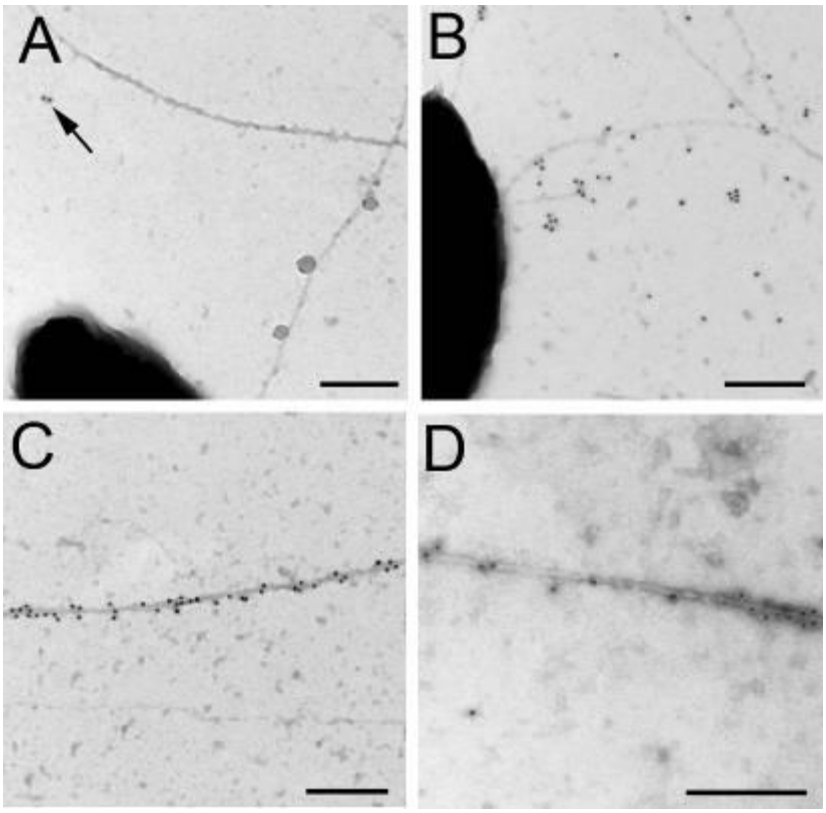

Figure 2. Immunogoldlabelling of HrpB2 (A and B) and the pilus structure protein HrpE (C and D) in the wildtype (A and C) bacteria and bacteria mutated in the secretion control protein hpaC (ΔhpaC; B and D). HrpE could be detected in pili of both strains whereas large amounts of HrpB2 were only found in the environment of pili of the ΔhpaC-strain.