Characterization of the interaction of an effector protein with the cysteine synthase OAS-TL

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1. Introduction

The plant pathogenic bacterium *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) causes bacterial spot disease on pepper and tomato and translocates more than 25 effector proteins via the type-III-secretion system into the plant cell cytoplasm (Fig. 1).

Effector proteins interfere with the plant pathways and manipulate them to the benefit of the pathogen.

One of these effector proteins is the XopC (Xanthomonas outer protein C). Bioinformatical analysis predicted two domains for XopC. Localization studies in *N. benthamiana* revealed that XopC is localized to the plant cell cytoplasm and the nucleus.

A yeast-two-hybrid screen with XopC identified a O-acetylserine-(thiol)-lyase (cysteine synthase) as putative interaction partner. These interaction was confirmed by CoIP and bimoleculare fluorescence complementation.

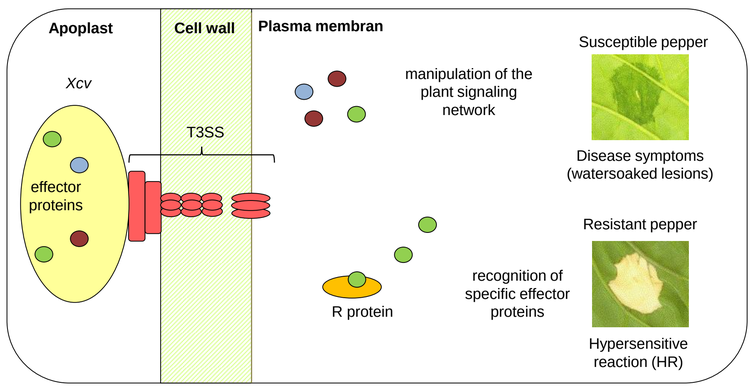

Fig. 1: Model of the interaction of *Xcv* + the plant cell. Plant phenotypes: 3 dpi (10^8 cfu/ml).

Fig. 3: Structural features of XopC. XopC consists of 834 amino acids (92 kDa) and has two predicted domains, a phosphoribosyltransferase domain and the second is a putative HAD-like hydrolase domain.

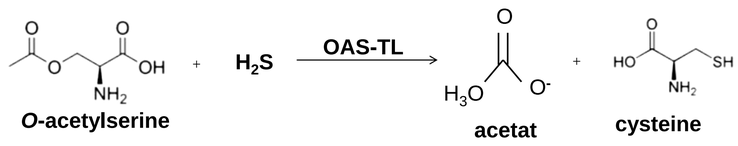

Fig. 4: Reaction of the biosynthesis of cysteine. Reduced sulfids and O-acetylserine were used to form acetat and the amino acid cysteine by the OAS-TL proteins.

2. XopC interacts with *Ca*OAS-TL *in vitro*

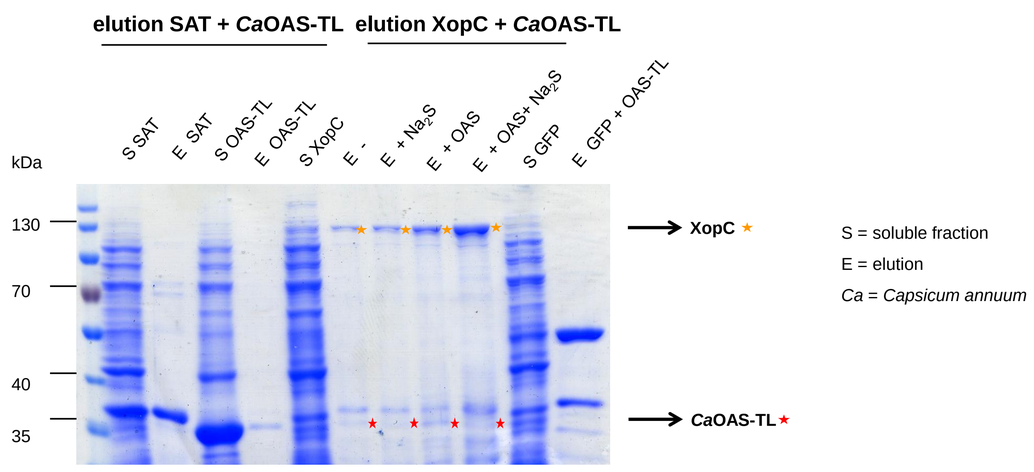

Fig. 4: XopC interacts *in vitro* with the pepper OAS-TL. GST-XopC was incubated with purified *Ca*OAS-TL protein in a GST pull down assay. Mass spectrometry confirmed that XopC but not GFP interact with the pepper OAS-TL *in vitro*.

3. XopC enhances the OAS-TL enzyme activity

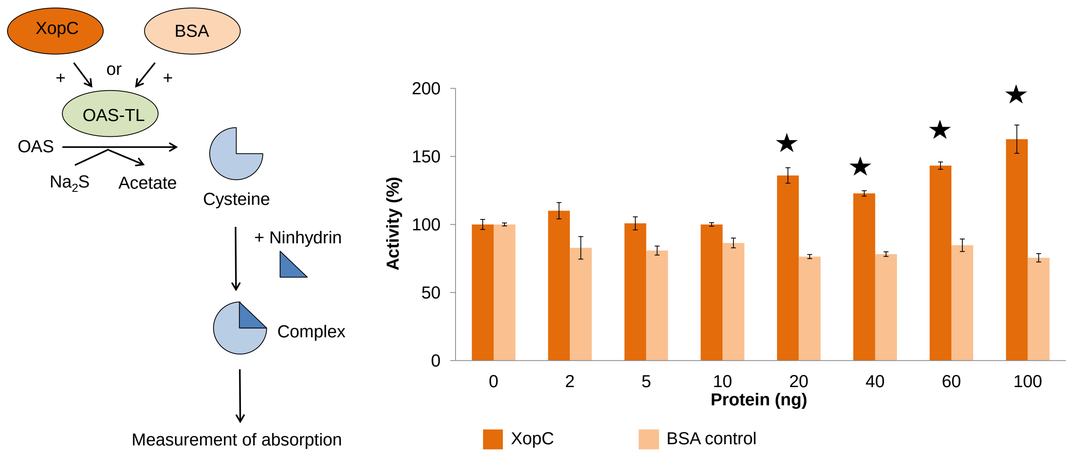

Fig. 5: OAS-TL activity assay *in vitro*. Purified XopC and CaOAS-TL were incubated with reduced sulfids and OAS as substrates. XopC and BSA were titrated in increasing amounts and produced cysteine can be measured by the use of acidic ninhydrin. The measured absorption is proportional to the enzyme activity. Three values were measured and averaged. ★: P < 0,05.

4. XopC impacts on cysteine levels *in planta*

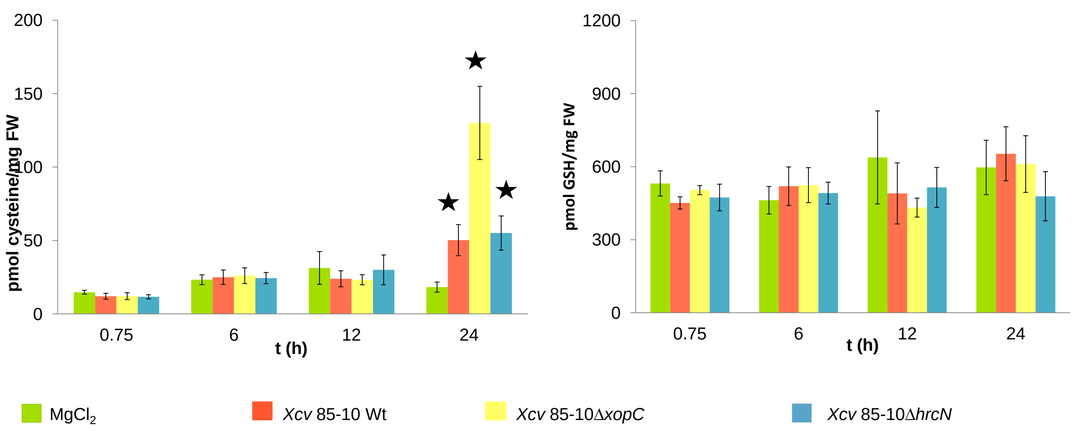

Fig. 6: Determination of cysteine and gluthathione levels. The *Xcv* strains were inoculated with 10^8 cfu/ml and harvested after 45 min, 6 h; 12 h and 24 h. A average of 5 replicates was used. After derivatization and determination of the metabolites via HPLC the concentrations were quantified. ★: P < 0,05.

5. Model of XopC action + open questions

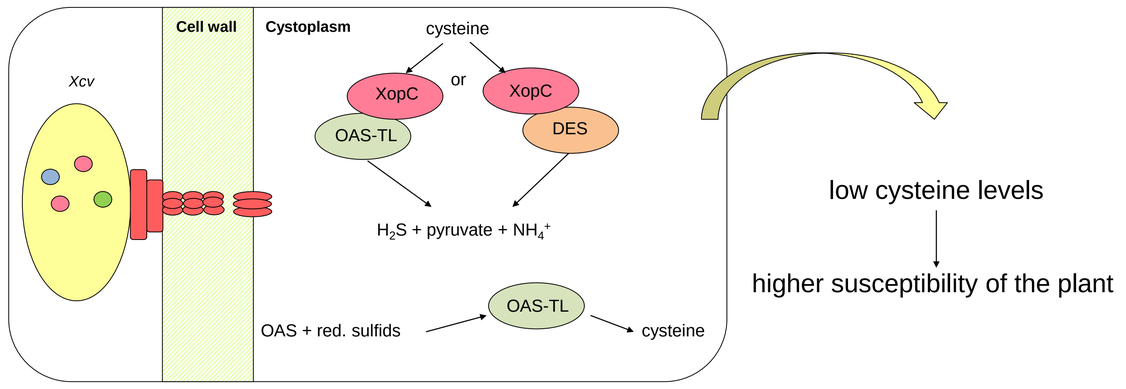

Fig. 7: Model of XopC activity in the plant cell. The interaction of XopC with the bifunctional OAS-TL protein or L-cysteine-desulfhydrase (DES) maybe degrades cysteine *in planta*.

1. Does XopC interact with other members of the β-substituted-alaninesynthase family?
2. Does this interaction lead to a degradation of cysteine *in vitro*?

Acknowledgement

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References

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[2] Büttner *et al*. (2003): *J Biotechnol; 106(2-3) 203-14*