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9-8 Heterologous expression of the viral proteinase of *Cherry leaf roll virus* (CLRV)

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INTRODUCTION

Cherry leaf roll virus (CLRV), a subgroup C-*Nepovirus*, belongs to the family of the *Secoviridae* (Sanfacon *et al.* 2009). The bipartite genome consists of positively orientated single-stranded RNA, which encodes for two polyproteins (P1 and P2). P1 harbors characteristic domains for a proteinase-cofactor (PCo), a helicase (Hel), a genome-linked protein (VPg), a proteinase (Pro), and an RNA-depending polymerase (Pol). P2 includes the movement protein (MP), the coat protein (CP) and a region at the 5`-end, that has not been functionally assigned by now (von Bargen *et al.* 2012). The polyproteins are processed to their functional units by the viral proteinase posttranslationally. *In-silico*-analysis of the full-length sequence revealed several putative processing-sites similar to already experimentally proven processing sites of related proteinases of nepoviruses like *Tomato ringspot virus* (ToRSV, Wang *et al.* 1999, Wang & Sanfacon 2000) and *Arabis mosaic virus* (ArMV, Wetzel *et al.* 2008). A prerequisite for the functional characterisation of viral gene-products is the elucidation of their processing to the mature subunits. Aim of this project is therefore the identification of the processing sites of the CLRV-proteinase after their heterologous expression in *E. coli* and native purification.

MATERIAL AND METHODS

In order to functionally characterize the proteinase of CLRV, it was expressed in *E. coli*. As the presence of the VPg was shown to affect the activity of the proteinase (Chisholm *et al*. 2001), the putative coding region of the proteinase, and a construct comprising the VPg and the proteinase were cloned into the expression vector pET28a (Novagen). *E. coli* expression strain BL21 DE3 was transformed and the proteins were heterologously expressed after induction with IPTG. Subsequently the proteins were purified under native conditions by Ni-NTA-agarose-affinity chromatography.

RESULTS AND OUTLOOK

The CLRV-Proteinease, as well as the construct consisting of the VPg and the proteinase were successfully expressed in *E. coli* and could be detected by SDS-PAGE and western blotting. Analogously, the genome-regions comprising the putative processing sites from both P1 (X1/PCo, PCo/Hel, Hel/VPg, VPg/Pro, Pro/Pol), and P2 (X3/X4, X4/MP, MP/CP) will be amplified from viral RNA via RT-PCR. After cloning and expression in *E. coli,* the proteins will be purified and subsequently subjected to *in vitro*-activity-assays as substrates for the proteinase. As a proof of principle, methods are presently being established using a construct consisting of the C-terminal part of the MP and the N-terminal part of the CP, including the putative processing site. The assumed proteolytic cleavage will be monitored by visualization of the processed substrate via SDS-PAGE and western blotting using the N- and C-terminal HIS-tags.

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