harpin

Xanthomonas axonopodis pv. glycines [Xag] 402 bp hpa1

PCR pET30a αKBL21

BHR-32 15.1kb

hpa1 [Xag] hpaG

harpin

harpin in X. campestris pv. campestris Xcc

harpin

harpin

harpin

1

1.1

1.1.1

28°C 28°C BL21

LB 37°C
e

20μg/mL

1.1.2

pET30a

Xag DNA

harpin
1.3 大豆斑疹病菌

1.4 HR HR HR NC89

1.5 TaKaRa Blast DNAStar

2.1 harpin harpin harpin

2.2 harpin harpin harpin

Fig.1 SDS-PAGE detection of an harpin protein expressed in E. coli
BL21 harboring a reconstructed plasmid pH73
M. Marker 1. BHR-3 2. BL21 as control.
Table 1 The properties of hpa1 gene and Hpa1 protein of *Xanthomonas axonopodis pv. glycines* compared with other plant *Xanthomonas* pathogens

<table>
<thead>
<tr>
<th>Source of hpa1 gene</th>
<th>Gene size/bp</th>
<th>Nucleotide identity/%</th>
<th>Amino acid number</th>
<th>Protein identity/%</th>
<th>Content of Gly/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xag</td>
<td>402</td>
<td>—</td>
<td>133</td>
<td>—</td>
<td>21.1</td>
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<tr>
<td>Xoe</td>
<td>411</td>
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<td>137</td>
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<td>143</td>
<td>59.4</td>
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<tr>
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<td>117</td>
<td>47.9</td>
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<tr>
<td>Xcc</td>
<td>366</td>
<td>51.4</td>
<td>121</td>
<td>29.8</td>
<td>13.2</td>
</tr>
</tbody>
</table>


2.3 *harpin*<sub>Xag</sub> (HPR-3) (BHR-3) (harpin<sub>Xag</sub>)

100°C 10min 0.1% (w/v) HR

harpin<sub>Xag</sub> 20ng/ml HR

HR harpin<sub>Xag</sub>

PMSF K Harpin<sub>Xag</sub> 7.1

10<sup>-5</sup> mmol/L 10<sup>-3</sup> mmol/L 10<sup>-4</sup> mmol/L

HR Harpin<sub>Xag</sub>

harpin<sub>Xag</sub> HR RNA

harpin<sub>Xag</sub> D

NahG Harpin<sub>Xag</sub>

Harpin<sub>Xag</sub> HR

Harpin<sub>Xag</sub>

Harpin
Cloning and characterization of an harpin-encoding gene from *Xanthomonas axonopodis* pv. *glycines* required for hypersensitive response on nonhost plant tobacco

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**Abstract** An *hpa1* gene was cloned into an expression vector pET30a + from the genomic DNA of *Xanthomonas axonopodis* pv. *glycines* Xag, the causal agent of soybean bacterial pustule, with degenerated primers by polymerase amplification reaction PCR. The gene product was extracted from the conjugate BLR-3 of BL2 DES with the recombinant vector pH3 after the engineering strain was induced by IPTG in LB medium. The SDS-PAGE gel showed that the gene product was 15.1 kD. The product was heat-stable 10 min at 100°C protease K sensitive and able to trigger hypersensitive response HR in common tobacco but was unable to elicit HR in *NahG* transgenic tobacco in which salicylic acid accumulation was abolished. Moreover, the HR elicitation of the protein in tobacco was dispelled by eukayotic metabolic inhibitors actinomycin D, cycloheximide and LaCl3. The 402 bp *hpa1* gene in this study putatively encoded a 133 ammonia acid protein of which glycine C was rich with 21.1 %. Sequence comparison indicated that the *hpa1* gene and its protein was 51.4 %~ 93.8 % identity with those of *Xanthomonas oryzae* pv. *oryzae* and other *Xanthomonas* species and pathogens. Alignments of harpin proteins of *Xanthomonas* genus displayed that the glycine-rich region with GGG-GG motif was variable. The comparison also showed that the harpin-encoding gene of Xag nominated here as *hpa1* Xag did not possess any similarity with that of *Erwinia amylovora* Pseudomonas syringae and *Ralstonia solanacearum* at nucleotide and protein levels. It is concluded that *hpa1* Xag gene encodes an harpin protein which elicits a typical HR in nonhost tobacco.

**Key words** *Xanthomonas axonopodis* pv. *glycines* Tobacco Hypersensitive response Harpin-encoding gene