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Lack of introns in putative parasitism factor gene, expansin (*exp*B2) from pale potato cyst nematode *Globodera pallida*

Odsotnost intronov v genu za ekspanzin (*exp*B2), verjetnem parazitskem dejavniku, pri beli krompirjevi ogorčici *Globodera pallida*

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Abstract: Expansins are a group of plant cell wall loosening proteins. In animals, functional expansin (EXPB1) has been discovered in the golden potato cyst nematode *Globodera rostochiensis*. In plant-parasitic nematodes expansins act as the parasitism factors or effectors. Molecular variability of another expansin (*exp*B2) gene was evaluated in the diverse populations of the *G. rostochiensis*. Comparison of the *exp*B2 gene structure in the two potato cyst nematode species, *G. rostochiensis* and *G. pallida*, revealed lack of all four introns in *exp*B2 gene of *G. pallida* species. Possible loss of introns in Gp-*exp*B2 is discussed.

Keywords: Cell wall degradation, *Globodera pallida,* effectors, expansin, intron, parasitism factor, plant-parasitic nematode, potato cyst nematode.

Izvleček: Ekspanzini so skupina proteinov, ki zrahlja rastlinsko celično steno. Pri živalih so odkrili funkcionalni ekspanzin (EXP1) pri vrsti rumena krompirjeva ogorčica, *Globodera rostochiensis*. Pri rastlinsko parazitskih ogorčicah ekspanzini delujejo kot parazitski dejavniki oz. efektorji. Pri *G. rostochiensis* je bila ovrednotena molekulska raznolikost dodatnega ekspanzinskega gena (*exp*B2). Primerjava strukture gena *exp*B2 pri dveh vrstah krompirjevih ogorčic, *G. rostochiensis* in *G. pallida*, je razkrila odsotnost vseh štirih intronov pri vrsti *G. pallida* v primerjavi z vrsto *G. rostochiensis*. Predvidevamo možnost izgube intronov pri Gp-*exp*B2.

Ključne besede: razgradnja celične stene, *Globodera pallida*, efektorji, ekspanzin, intron, parazitski dejavnik, rastlinsko parazitske ogorčice

Introduction

Expansins are a group of proteins that operate by loosening non-covalent interactions between components of the plant cell wall making the individual components vulnerable to attack by other cell wall degrading enzymes (Cosgrove et al. 2000). These proteins were thought to be specific to plants; however an active expansin EXPB1 has unexpectedly been identified in the plant-parasitic nematode, golden potato cyst nematode *Globodera rostochiensis* (Woll.) Behrens (Qin et al. 2004). The potato cyst nematodes (PCN) *G. rostochiensis* and *G. pallida* (Stone) Behrens are plant-parasitic nematodes which parasitize different Solanaceous plant species. PCN pose a serious threat to potato

production worldwide, and they are subject to strict quarantine regulations in many countries. In Slovenia *G. rostochiensis* has spread in the last decade (Širca et al. 2010), while *G. pallida* has been first detected in Slovenian soil just recently (Širca et al. 2012).

When PCN invade a plant, they produce a mixture of lytic enzymes and expansins in their oesophageal glands and secrete them through the stylet into the plant. These proteins assist in the migration of infective juveniles through the host plant's tissues, and in the feeding site formation. Additionally, the host plant's own expansin genes are up-regulated upon nematode infection (Fudali et al. 2008). Expansins B1 and B2 were determined in the EST analysis of *G. rostochiensis,* while only expansin B1 was found in the *G. pallida* EST database (Popeijus et al. 2000, http://www.nematodes.org/nembase4/overview.shtml). Molecular variability of *exp*B2 gene was evaluated in the diverse populations of the *G. rostochiensis* (Gerič Stare et al. 2012).

The aim of this study was to check for the possible presence of the *exp*B2 in *G. pallida* and to determine its structure.

Materials and methods

DNA was extracted from 10 cysts of the *G. pallida* population. DNA extraction, amplification of *exp*B2 gene, cloning of the amplicon, isolation of pDNA, sequencing and sequence analysis were performed as previously described in detail by Gerič Stare et al. (2012) for the orthologous *exp*B2 gene in *G. rostochiensis* (*Gr-expB2*). Primers were designed based on the *Gr-expB2* mRNA sequence (AJ311902) coding for the putative functional expansin.

Results

The primer set designed for the *expB2* gene of *G. rostochiensis* yielded a much shorter PCR amplicon with the *G. pallida* genomic DNA (543 bp in *G. pallida* vs. 1.129 – 1.153 bp in *G. rostochiensis*). Sequence analysis revealed high homology to previously determined Gr-*exp*B2 precursor (AJ311902) by BLASTN with E value 0.0. Alignment of this *G. pallida* sequence with the previously determined genomic sequences of Gr-*exp*B2 (FJ705444, GQ152151 – GQ152166, GQ152168 – GQ152288) revealed lack of all four introns. Due to the high homology of the coding region of the Gr-*exp*B2 gene, this sequence was designated Gp-*exp*B2. Gp-*exp*B2 shared 99.6% identity with Gr-*exp*B2 cDNA (GQ152150). Further, no highly homologous sequence to Gp-*exp*B2 could be found in the *G. pallida* genome sequence (http://www.sanger.ac.uk/resources/downloads/helminths/globodera-pallida.html), although there are several sequences similar to expansin, except for including introns. The sequences Gp-*exp*B2 reported here was deposited at Genbank with the accession number GQ152167.

Discussion

In the database of *G. pallida* EST sequences (http://www.nematodes.org/nembase4/index.shtml) there are no expansin B2 related sequences. Nonetheless, we have determined orthologous sequence using the primer set developed for Gr-*exp*B2 in a PCR with *G. pallida* gDNA. Gp-*exp*B2 sequence showed high similarity to exons of determined Gr-*exp*B2 sequences but lack of all four introns found in *G. rostochiensis*. Closely related species usually possess conserved introns, but there are also examples where introns are present in one species but not in closely related one (Kent and Zahler, 2000). From an alignment of two sequences it is not possible to tell whether the introns are being lost or gained.

One hypothesis is that introns may be lost during repair of double-stranded breaks in DNA helix in a repair mechanism involving reverse transcription of mRNA and reintegration of the synthesized cDNA into the genome by homologous recombination. Another example of a gene where this process might have happened is a gene for β-1,4-endoglucanases in *Heterodera glycines* (Hg-*eng5*), a parasitic factors of plan-parasitic nematodes that contains no introns (Gao et al. 2004, Kyndt at al. 2008). On the other hand, a recent horizontal gene transfer event from a prokaryote was suggested for lack of introns in Hg-*eng*5 (Gao et al. 2004). Furthermore, formation of genes without intron from paralogous genes with introns

in eukaryotes might arise from gene duplication process involving reverse transcription of mRNAs with recombination of the synthesized cDNA in the genome (Boudet et al. 2001).

Conclusion

Comparison of orthologous *exp*B2 gene in potato cyst nematodes revealed lack of all four introns in *G. pallida* compared to *G. rostochiensis*.

Povzetek

Ekspanzini so proteini, ki z zrahljanjem nekovalentnih vezi pomagajo pri razgradnji rastlinske celične stene. Pri živalih so odkrili funkcionalni ekspanzin (EXP1) pri vrsti rumene krompirjeve ogorčice *Globodera rostochiensis* (Nematoda). Pri rastlinsko parazitskih ogorčicah ekspanzini delujejo kot parazitski dejavniki oz. efektorji. Pri *G. rostochiensis* je bila ovrednotena molekulska raznolikost dodatnega ekspanzinskega gena (*exp*B2). Z uporabo začetnih oligonukleotidov predhodno razvitih za Gr-*exp*B2 smo določili prisotnost gena *exp*B2 tudi pri sorodni vrsti bele krompirjeve ogorčice *G. pallida*. Primerjava strukture gena *exp*B2 je razkrila odsotnost vseh štirih intronov pri Gp-*exp*B2 v primerjavi Gr-*exp*B2. Do izgube intronov pri Gp-*exp*B2 bi lahko prišlo na različne načine. Prvi je mehanizem popravljanja poškodb dvoverižne DNA, ki vključuje obratno prepisovanje mRNA v cDNA in reintegracijo le te v genom s homologno rekombinacijo. Drug možen mehanizem je nedaven horizontalni prenos gena iz prokariontov. Tretja možnost nastanka genov brez intronov pri evkariontih je z podvojitvijo paralognih genov z introni v procesu, ki vključuje obratno prepisovanje mRNA in rekombinacijo tako nastale cDNA v genom.

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