Research Article

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Homology modelling and docking studies in an odorant binding protein from palm weevil

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Abstract

Knowledge-based homology modeling and substrate-docking experiments as well as structure and sequence comparisons were performed in an odorant binding protein RpalOBP4 from the palm weevil, *Rhynchophorus palmarum*. Initially, alignment for similarity studies was undertaken using BLAST. Interpro-analysis based PROSITE and Pfam databases search strongly recommend the protein in the PF01395 cluster of PBP-GOBP family. Physicochemical properties of the selected proteins were determined using the PROTPARAM tools. The Kyte and Dolittle hydropathy plot revealed that RpalOBP4 proteins are amphipathic. Secondary structure prediction was of a-helices. Function assignments were made based on the structural homologues (2ERB, 2H8V) identified for the test protein. 3-D structure was generated based on the secondary analysis. The structure thus obtained was assessed using PROCHECK. The distribution of residues in the most favoured region of the Ramachandran plot for RpalOBP4 was greater than 90%. Ligand docking of the RpalOBP4 model with five substrates was also conducted.

Keywords: in silico prediction, molecular modelling, odorant binding protein

Introduction

Structural genomics is expected to elucidate many experimentally determined proteins structures. Since solving a protein structure by NMR or crystallography remains a long and expensive effort, constructing 3-D models based on structures of homologous proteins is an alternative approach.

The behaviour of insects, in majority of cases, is driven by olfactory cues hence, the molecules and processes involved in the olfactory transduction pathway are ideal targets to device pest management strategies. Insect odorant binding proteins (OBPs) act as carriers of odour molecules to olfactory receptors. They aid in transport of lipophilic odorants entering through the pores in sensilla to membrane-bound receptors in the olfactory neurons (Vogt *et al.*, 1999). Multiple OBPs have been identified in single species and have been shown to associate differentially with functionally distinct classes of olfactory sensilla (Laue and Steinbrecht, 1997; Vogt *et al.*, 1999). OBPs have been identified from Lepidoptera, Diptera, Coleoptera and Hymenoptera. The odorant-binding protein RpalOBP4 has been isolated by Meillour *et al.* (2004) from the palm weevil, *Rhynchophorus palmarum* that has an extensive worldwide distribution. It is a pest on oil palm, coconut and aids as a vector for red ring nematode.

Homology modelling provides a powerful new tool for uncovering olfactory genes and allows inferences to be made regarding the potential sites and function encoded olfactory proteins. The functional assignment through structural exploration of these proteins would be of immense use for olfactory studies. Molecular docking studies can provide a picture of interaction of ligands with the odorant binding protein. Effectively scored candidate ligands can be used for devising control strategies for *Rhynchophorus palmarum*. The present investigation summarizes the work on structural features and the search for good candidates for bio-control of the putative odorant binding protein RpalOBP4 of *Rhynchophorus palmarum* employing *in silico* tools.

Materials and Methods

Datasets

Sequence of OBP from the palm weevil *Rhynchophorus palmarum* (RpalOBP4; accession number: AAO64978) and other sequences were retrieved

to potato dextrose broth (PDB) and incubated at 25°C on an orbital shaker (100 rpm) for 4 days. Extraction and purification of the total genomic DNA were carried out following the modified CTAB protocol (Saha *et al.*, 2000).

Twenty arbitrary decamer primers (Operon technology Inc., USA) were selected based on our earlier studies (Roy *et al.*, 2006) for PCR amplifications of *Alternaria* and *Corynespora* cultures. Amplifications were performed in a total volume of 25 µl by mixing 50 ng of template DNA with 10 picomoles of single primer, 0.2 mM of each dNTPs, 0.7 units of *Taq* DNA polymerase (GE Heathcare, UK) and 2.5 µl of 10x DNA polymerase buffer. Amplifications were performed in a thermal cycler with an initial denaturation step at 94°C for 3 min., followed by 40 cycles of 30 sec. at 94°C, 1 min. at 37°C and 2 min. at 72°C with a final extension at 72°C for 7 min. Amplified products were analysed along with a DNA marker, as molecular size reference, by electrophoresis on a 1% agarose gel in 1x TBE buffer, stained with ethidium bromide (0.5 g/ml), visualized with ultraviolet light and photographed using Gel-documentation system (Bio-Rad Laboratories Inc., USA).

Two isolates each of *Alternaria* and *Corynespora* were chosen to develop pathogen-specific SCAR marker for *Alternaria*. After performing RAPD-PCR with selected operon primers, the amplified product was electrophoresed and bright, consistent and specific bands existing only in *Alternaria* was selected for conversion into Alternaria-specific SCAR marker. Based on differential banding pattern between *Alternaria* and *Corynespora*, nine RAPD primers were selected and used for further analysis. Six RAPD fragments $OPA17_{800}$, $OPA17_{900}$, $OPA19_{1100}$, $OPAB11_{1000}$, $OPAC5_{700}$ and $OPAF17_{1200}$ were selected for conversion to SCAR markers (the names of the fragments chosen indicate the primer with which the fragment was amplified and the size of the amplified product is given as subscript). The intensity of the amplification products and their good separation from neighbouring RAPD bands facilitated their isolation and cloning.

The selected RAPD marker bands from *Alternaria* were excised from the agarose gel using sterile blades and purified using GFX-Gel band purification kit (GE Healthcare, UK). The purified fragments were cloned using the pGEM-T Eazy vector system (Promega Corporation, USA). Subsequently, the positive clones were selected by blue white screening and confirmed by colony PCR using vector directed primers. This was followed by sequencing of the positive clones in both forward and reverse orientations. From each sequenced RAPD marker sequence, oligonucleotides to be tested as SCAR primers were designed based on the forward and reverse sequences. The presence of the RAPD primer was checked at both ends of the sequences and primers were designed consisting mainly of the 10 bases of the RAPD primer completed by the following 3' base sequence to design a 20-23 mer primer with an approximate Tm value of 60°C. Primers were custom synthesized by MWG Biotech, India (Table 1).

Genomic DNA from two representative isolates of *Alternaria* and *Corynespora* were used as templates to optimize annealing temperature. PCR thermal programme was as follows: initial denaturation at 94°C for 3 min. followed by 35 cycles of 94°C for 30 sec., annealing at 58-62°C for 1 min. depending on the Tm of the primers and 72°C for 2 min. with a final extension at 72°C for 7 min. Each PCR reaction contained 50 ng of genomic DNA template, 0.7 units of *Taq* DNA polymerase, 0.2 mM of dNTP, 10 pmol each of forward and reverse SCAR primers, 2.5 µl of 10x PCR buffer with 1.5 mM $MgCl_2$ in a final volume of 25 µl. The products were visualized and photographed as described earlier. The primer-pairs were tested for both sensitivity and specificity. To determine sensitivity, serial dilutions of the *Alternaria* genomic DNA were prepared with TE (10:0.1) buffer and added at concentrations of 1 to 50 ng per reaction to check for amplification of the fungal DNA at its least concentration. To determine specificity,

Table 1. Details of SCAR markers developed from the RAPD primers for identification of *Alternaria*

SCAR marker for the genus	RAPD fragment converted to SCAR	SCAR primers	Nucleotide sequence (5' to 3')	Tm(°C)
	$OPAB11_{1000}$	SCAR-1F	GTGCGCAATGGGTGTTGTAGC	61.8
	$OPAB11_{1000}$	SCAR-1R	GTGCGCAATGACGGTTAACAGT	60.3
Alternaria	$OPA17_{800}$	SCAR-14F	GACCGCTTGTGGAGGGCATG	63.5
	$OPA17_{800}$	SCAR-14R	GACCGCTTGTTACCTACCCATT	60.3
	$OPA17_{900}$	SCAR-15F	GACCGCTTGTCAATTAACTGAC	58.4
	$OPA17_{900}$	SCAR-15R	GACCGCTTGTGGTATGAGCA	59.4

the primer-pairs were tested with the fungal DNA from both the pathogens *Alternaria* and *Corynespora*.

Results and Discussion

Two highly variable representative isolates each of *Alternaria* and *Corynespora* were chosen based on our earlier studies for the development of pathogen-specific SCAR marker for *Alternaria*. After RAPD-PCRs, DNA electrophoresis revealed polymorphic bands between these organisms using nine primers namely, OPA4, OPA7, OPA8, OPA17, OPA19, OPA20, OPAB11, OPAC5 and OPAF17. Intense, unique bands of size 0.5 to 1.5 kb, specifically present only in *Alternaria* were selected (Fig. 1) as they were expected to serve as marker. Six RAPD fragments $OPA17_{800}$, $OPA17_{900}$, $OPA19_{1100}$, $OPAB11_{1000}$, $OPAC5_{700}$ and $OPAF17_{1200}$ were selected for conversion to SCAR markers.

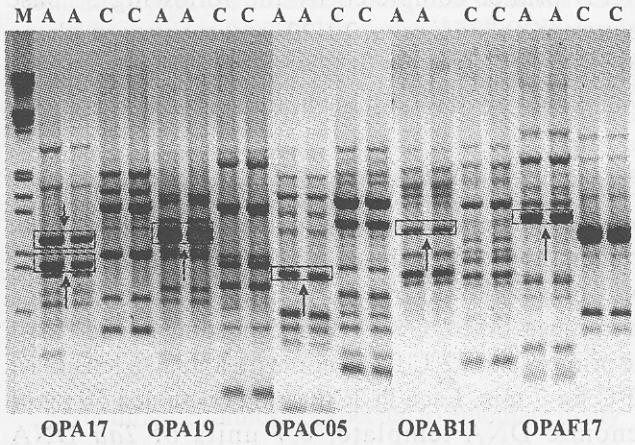

Fig. 1. Amplification patterns obtained with RAPD primers using template DNA from two isolates each of *Alternaria* (A) and *Corynespora* (C).
Arrow indicates polymorphic band that was cloned and sequenced for the development of *Alternaria*-specific primer pair.
M: Molecular weight marker (l-DNA *Eco* RI + *Hind* III double digest).

Using the sequence information of the cloned specific RAPDs, six pairs of putative SCAR primers were designed and tested for their specificity in amplification. Three pairs of primers produced multiple bands and did not reveal polymorphism between the two fungi. Multiple bands produced by these primer-pairs could be attributed to the fact that the original polymorphisms with the 10-base RAPD primers were due to small mismatches at the priming sites resulting in no amplification products at this site, whereas the longer SCAR primers (20-23 bases) were not affected by small mismatches at the priming sites. The positional shifts observed for these markers as compared to the original RAPD markers are due to the higher experimental stringency applicable to SCAR markers. They were therefore deemed to be unsuitable for discrimination (Fig. 2). However, the additional band could be avoided by increasing the annealing temperature. Finally, two SCAR primer-pairs generating SCAR markers: SCAR1 and SCAR14, derived from RAPDs $OPAB11_{1000}$ and $OPA17_{800}$ respectively, showed unique amplicons of expected sizes matching perfectly with the original RAPDs from which they were derived (Table 1; Fig. 3). These two SCAR markers were therefore successfully used to detect *Alternaria* causing leaf spot disease in rubber.

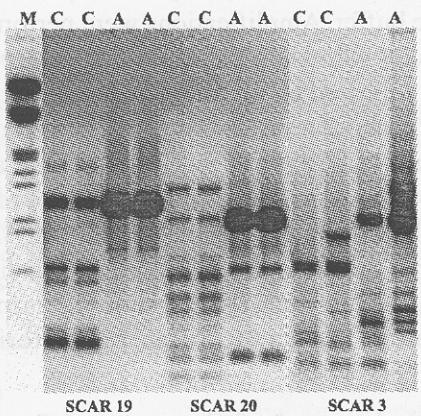

Fig. 2. SCAR products amplified using the SCAR primers revealing the specific amplification of the desired band in *Alternaria* (A) isolates along with a few minor bands in *Corynespora* (C) isolates.
M – Molecular weight marker (l-DNA *Eco* RI + *Hind* III double digest).

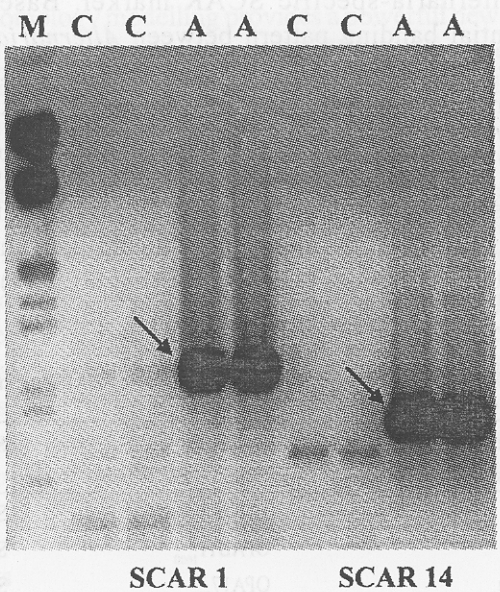

Fig. 3. Banding pattern of *Alternaria* isolates showing a distinct and reproducible band only in *Alternaria* (A) and not in *Corynespora*
M: Molecular weight marker (l-DNA *Eco* RI + *Hind* III double digest).

Sensitivity tests with serial dilutions of total *Alternaria* DNA proved that the limit of detection (*i.e.*, the lowest concentration of total genomic DNA from which the target fragment got amplified) was 1 ng in a standard PCR reaction volume of 25 µl (Fig. 4). This could indicate that these primers would detect the pathogen by PCR, even in their early stage of symptom development. Hence, this PCR test could also be of great importance to check nursery plants as a means of preventing the spread of the pathogen into disease-free areas. Specificity of the SCAR primers with genomic

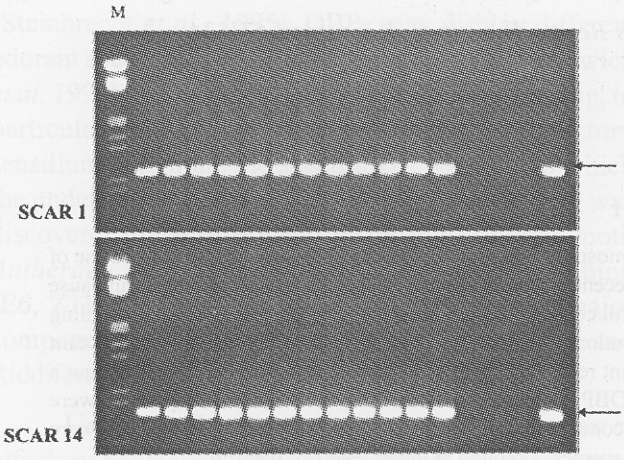

Fig. 4. Limit of detection of the *Alternaria* SCAR primers with varying concentrations of *Alternaria* DNA.
Positive control with *Alternaria* DNA alone as well as negative control with *Corynespora* DNA are also shown. Amplifications performed directly with the diseased sample also indicated the efficiency of the SCAR primer. Arrow indicates specific SCAR marker.
M: Molecular weight marker (l-DNA *Eco* RI + *Hind* III double digest)

DNA from both the pathogens also showed that the SCAR markers directed the amplification of the specific fragment only in *Alternaria*. The primers were tested against host genomic DNA for its interference with the amplification of the fungal DNA. It was observed that the SCAR primers efficiently amplified the fungal DNA even in the presence of plant DNA. This observation is considered to be significant as the diseased sample can directly be used in the detection of these pathogens without establishing pure cultures of the respective pathogen. Due to the specificity and sensitivity of the primers, they could be useful in the bio-PCR technique by speeding up identification of plant material infected by these pathogens.

The present work proves SCAR marker to be an efficient way for identification of *Alternaria* causing leaf disease in rubber. The results encourage the application of this PCR-based identification method in setting up a reliable diagnostic assay for their detection from infected rubber plantations as early detection is essential for controlling the spread of the pathogen thereby making this technique appropriate for diagnostic purposes.

References

Balakrishnan, P. and Prameswaran, P.2007. Understanding Economic Growth in India: A Prerequisite. *Economic and Political Weekly.*

Akkurt, M., Welter, L., Maul, E., Topfer, R. and Zyprian, E. 2007. Development of SCAR markers linked to powdery mildew (*Uncinula necator*) resistance in grapevine (*Vitis vinifera* L. and *Vitis* sp.). *Mol. Breed.* **19**: 103-111.

Kethidi, D.R., Roden, D.B., Ladd, T.R., Krell, P.J., Retnakaran, A. and Feng. Q. 2003. Development of SCAR markers for the DNA-based detection of the asian long-horned beetle, *Anoplophora galbripennis* (Motschulsky). *Arch. Insect Biochem. Physiol.* **52**: 193-204.

Paran, I. and Michelmore, R.W. 1993. Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. *Theor. Appl. Genet.* **85**: 985-993.

Pryor, B.M. and Gilbertson, R.L. 2000. A PCR-based assay for detection of *Alternaria radicina* on carrot seed. *Plant Dis.* **85**: 18-23.

Roy, C.B., Zachariah, C.A., Jacob, C.K. and Saha, T. 2006. First report of leaf blight caused by *Alternaria alternata* on *Hevea brasiliensis* in India. *J. Plantn. Crops* **34**: 449-454.

Rugienius, R., Siksnianas, T., Stanys, V., Gelvonauskiene, D. and Bendokas, V. 2006. Use of RAPD and SCAR markers for identification of strawberry genotypes carrying red stele (*Phytophthora fragariae*) resistance gene *Rpf1*. *Agronomy Res.* **4**: 335-339.

Saha, T., Kumar, A., Sreena, A.S., Joseph, A., Jacob, C.K., Kothandaraman, R. and Nazeer, M.A. 2000. Genetic variability of *Corynespora cassiicola* infecting *Hevea brasiliensis* isolated from the traditional rubber growing areas in India. *Ind. J. Nat. Rub. Res.* **13**: 1-10.

Ye, Q., Qiu, Y., Quo, Y., Chen, J., Yang, S., Zhao, M. and Fu, C. 2006. Species-specific SCAR markers for authentication of *Sinocalycanthus chinensis*. *J. Zhejiang Univ.* **7**: 868-872.