Cloning of *Sclerotium rolfsii* lectin gene and its nematicidal activity

Several fungal lectins have been purified and characterized. Many of them play a biological role in antitumour, immunomodulatory and insecticidal activities. *Sclerotium rolfsii*, a soil-borne plant pathogenic fungus produces lectins that act as signalling molecules in interaction with antagonists[1–3] and germination of sclerotial bodies[4]. A 17 kDa *S. rolfsii* lectin (SRL) agglutinated trypsinized rabbit and human erythrocytes and displayed a strong binding to disaccharide Gal βl $\rightarrow$ 3 GalNAc-α- (Thomsen Friedenreich antigen)[5] that is over-expressed in human tumour cells. SRL shared common structural topology, glycan specificity and carbohydrate-binding sites with *Xerocomus chrysenteron* lectin (XCL), a fungal lectin possessing antiproliferative and insecticidal activity[6]. Considering the use of such fungal lectins in pharmaceutical and crop improvement applications, an effort was made to clone and characterize a lectin gene from *S. rolfsii*, and to express in *Escherichia coli*. Heterologously expressed lectin was checked for nematicidal activity using a common root knot nematode, *Meloidogyne incognita*.

S. rolfsii culture was obtained from the Department of Plant Pathology, UAS, Dharwad. It was maintained on potato dextrose agar slants containing 5% dextrose. Whole genomic DNA was isolated from mycelia of *S. rolfsii* by following CTAB method with modifications[7]. Two degenerate primers (forward: 5′ATGACNTAYAARATHAC3′ and reverse: 5′NCCDATDATNARRTT3′) designed based on N (Met–Thr–Tyr–Lys–Ile–Thr–Val–Arg–Val–Tyr) and C-terminal (Asn–Asn–Leu–His–Ala–Asn–Leu–Ile–Ile–Gly) amino acid sequence of SRL[8] were employed in PCR. The product was cloned into pTZ57R/T, and transferred to *E. coli* DH5α to get pCR12 clone. Insert was sequenced at Bangalore Genei, Bangalore. A 548 bp sequence contained an open reading frame (ORF) of 429 bp corresponding to a polypeptide of 142 residues with a molecular weight of 16 kDa. Deduced polypeptide had 76.1% sequence identity with SRL, and showed 34 non-identical amino acids over 142 residues. However, it was found that both primary (Tyr28, Ala29, Ser48, Gly49, His71, Asn72, Tyr73, Arg106) and secondary (Asp78, Ile79, Thr81, Arg102, Tyr113, Val115) carbohydrate-binding sites remained unchanged (Figure 1). We named the polypeptide as SRL-like lectin, and the gene coding for it as *srl1*. The homology search (BLASTp) for the amino acid sequence of SRL-like lectin revealed that it belonged to a class of fungal fruit body lectin. Multiple alignment of the SRL-like lectin showed significant homology (46–76% identity) with fungal lectins of *Agaricus bisporus*, *Arthrobothrys oligospora*, *Pleurotus cornucopiae*, *X. chrysenteron*, *Podospora anserine* and *Paxillus involutus*.

For heterologous expression of *srl1* in *E. coli*, an expression vector, pCR29 was constructed by re-amplifying only the ORF from genomic DNA of *S. rolfsii* using forward (5′TCAT CCATGG ATGACTTATAAGATTACCGT3′) and reverse (5′TGA GGATCC TCACCCGATAATGACGTT3′) primers containing *Nco*I and *Bam*HI sites respectively, and ligating the purified product with pET-32b(+) (Novagen #69016-3). Care was taken to have His-tag fusion at NH_2 end of the expressed polypeptide. pCR29 was transferred to *E. coli* expression strain, BL21(DE3)pLysS (Novagen #69388-3). Expression of the lectin was induced by adding isopropyl-β-D-thiogalactopyranoside (IPTG) at a final concentration of 1 mM. Cells were harvested by centrifugation, washed with 0.5% (w/v) NaCl and resuspended in 500 µl of phosphate buffer saline (PBS; 50 mM, pH 7.2). The cell suspension was sonicated on ice for 1 min for six times at an interval of 1 min. Contents were centrifuged at 13,000 rpm at 4°C for 20 min, and the supernatant containing the total protein was collected. Further purification of the His-tag fused lectin was done with Ni-NTA columns (QIAGEN NI-NTA spin kit, cat. no. 31314) according to manufacturer's protocol.

Molecular size of the SRL-like lectin was determined by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) by following the procedure of Sambrook and Russell[7]. Samples were loaded on to 10% polyacrylamide gel placed in a Mini-PROTEAN® Tetra cell unit (BIO-RAD). Gel was stained with commassie brilliant blue and the excess dye was removed by repeated washing every 2–3 h with de-staining solution till blue colour band appeared. *E. coli* expressed fusion protein showed a band of ~19.5 kDa (Figure 2), which is smaller than the expected size of 33.4 kDa (16 kDa of SRL-like lectin and 17.4 kDa of the tag (Trx-S-His)). Such a variation in molecular size could be due to any post-translational modifications[9]. SRL-like lectin differed in molecular weight from previously reported three (45[1], 55[3] and 60[3] kDa) lectins of *S. rolfsii*. Purified lectin was subjected to haemagglutination assay[10] by the serial two-fold dilution technique of Liener and Hill[11], and sugar specificity was determined by Hapten inhibition assay[12]. SRL-like lectin could agglutinate trypsinized rabbit and human erythrocytes, and possessed sugar specificity for asialofetuin and mucin like that of SRL, indicating that it could be an isoform of SRL. Such lectin isoforms are common in plants and fungi[6,13].

Because of its considerable homology (56% identity, 73% similarity) to an insecticidal lectin (XCL), SRL-like lectin was considered for the juvenile inhibition assay with *M. incognita*, a major plant root knot nematode. Juvenile

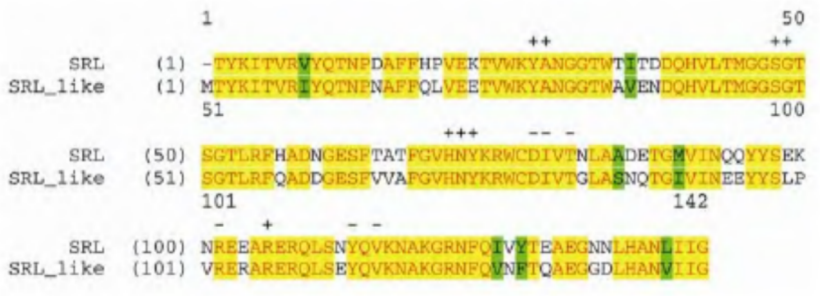

Figure 1. Alignment of SRL-like lectin with SRL. Every 50 residues in SRL-like lectin are numbered. Primary (+) and secondary (–) carbohydrate binding sites in SRL-like lectin are indicated.

Table 1. *Meloidogyne incognita* juvenile mortality (%) caused by SRL and *E. coli* expressed SRL-like lectin

Time (h)	Purified SRL (0.90 mg)		*E. coli* protein with SRL-like lectin (20.04 mg)		*E. coli* protein without SRL-like lectin (0.65 mg)	PBS	Distilled water
	1 : 4	1 : 19	1 : 4	1 : 10			
3	0	0	0	0	0	0	0
6	1.8	0	0	0	0	0	0
12	12.5	7.5	0	0	0	0	0
24	40.0	12.0	20.0	8.0	0	0	0
48	48.0	36.0	50.0	22.0	0	0	0

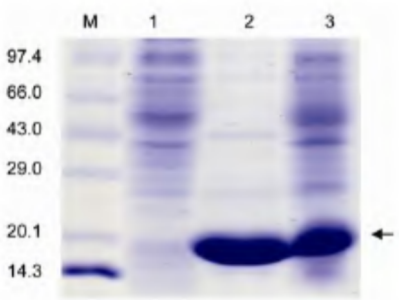

Figure 2. SDS–PAGE for *E. coli* expressed SRL-like lectin. M, Protein molecular weight marker (medium range); 1, Crude protein from *E. coli* carrying pET-32b(+); 2, His-tag purified SRL-like lectin; 3, Crude protein from *E. coli* carrying pCR29.

inhibition test for *M. incognita* was carried out by incubating at least 50 juveniles (stage 2; J2) in two dilutions (1 : 4 and 1 : 10) of 20.04 mg total protein from *E. coli* expressing SRL-like lectin. Two dilutions (1 : 4 and 1 : 19) of purified SRL (0.90 mg) from sclerotial bodies[14] were also employed for the assay. Crude protein (0.65 mg) from *E. coli* BL21(D3)-pLysS containing pET-32b(+), PBS and distilled water were used as negative controls. Number of inactive juveniles was recorded at 3, 6, 12, 24 and 48 h and expressed in percentage. Juveniles not regaining activity even after 2 h in water were considered dead. Both purified SRL and total protein with SRL-like lectin showed nematicidal activity against root knot nematode. With SRL, the mortality was noticed after 6 h of incubation, it increased with time and concentration (Table 1). Maximum mortality of 48% was noticed with 1 : 4 dilution after 48 h. Total protein containing SRL-like lectin showed mortality after 24 h with both 1 : 4 and 1 : 10 dilutions. Maximum of 50% mortality was seen in 1 : 4 dilution after 48 h. Juveniles placed in water and PBS did not show any mortality.

Two plant lectins, viz. concanavalin A (ConA) lectin of *Canavalia ensiformis* and *Galanthus nivalis* agglutinin (GNA) are known to be nematicidal[15,16]. To our knowledge, this is the first report on nematicidal activity of a fungal lectin, though lectin-mediated nematode trapping is established for some fungi[17]. Like in insects, glycoproteins found along the digestive tract of nematodes might be exposed to a diet containing lectin, and its binding to such receptors[15,16] may have either an anti-feedant effect, or result in mortality or retarded growth[18]. Stylet of *M. incognita* displays a size exclusion limit of 28 kDa[19]. SRL-like lectin (16 kDa) might get ingested by the nematode. Alternatively, lectin can bind to glycoproteins localized on the surface of the nematodes, or chemoreceptors present in the amphids and amphidial secretions, and interfere with the sensory perception and the ability to establish feeding cells[20]. Such surface receptors have been characterized from the nematode, *Panagrellus redivivus*[21]. Availability of *srl1* gene therefore enables testing nematicidal activity *in vivo*, in addition to express SRL-like lectin for studies on pharmaceutical applications.

1. Inbar, J. and Chet, I., *Microbiology*, 1994, **140**, 651–657.
2. Barak, R., Elad, Y., Mirelman, D. and Chet, I., *Phytopathology*, 1985, **75**, 458–462.
3. Barak, R. and Chet, I., *J. Appl. Bacteriol.*, 1990, **69**, 101–112.
4. Swamy, B. M., Bhat, A. G., Hegde, G. V., Naik, R. S., Kulkarni, S. and Inamdar, S. R., *Glycobiology*, 2004, **14**, 951–957.
5. Wu, A. M., Wu, J. H., Tsai, M. S., Hegde, G. V., Inamdar, S. R., Swamy, B. M. and Herp, A., *Life Sci.*, 2001, **69**, 2039–2050.
6. Trigueros, V. *et al.*, *Biochim. Biophys. Acta*, 2003, **1621**, 292–298.
7. Sambrook, J. and Russell, D. W., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, USA, 2001.
8. Leonidas, D. D. *et al.*, *J. Mol. Biol.*, 2007, **368**, 1145–1161.
9. Yoon, S., Kingsman, S. M., Kingsman, A. J., Wilson, S. A. and Mitrophanous, K. A., *J. Gen. Virol.*, 2000, **81**, 2189–2194.
10. Lis, H. and Sharon, N., *Chem. Rev.*, 1998, **98**, 637–674.
11. Liener, I. E. and Hill, E. G., *J. Nutr.*, 1953, **49**, 609–620.
12. Hariharan, K. and Rao, G., *Proc. Indian Acad. Sci.*, 1978, **87B**, 63–66.
13. Hirano, K., Teraoka, T., Yamanaka, H., Harashima, A., Kunisaki, A., Takahashi, H. and Hosokawa, D., *Plant Cell Physiol.*, 2000, **41**, 258–267.
14. Swamy, B. M., Hegde, G. V., Naik, R. S. and Inamdar, S. R., *Lect. Biol. Biochem. Clin. Biochem.*, 2001, **15**, 45–55.
15. Ripoll, C., Favery, B., Lecomte, P., Van Damme, E., Peumans, W., Abad, P. and Jouanin, L., *Plant Sci.*, 2003, **164**, 517–523.
16. Marban-Mendoza, N., Jeyaprakash, A. and Bjansson, H., *J. Nematol.*, 1987, **19**, 331–335.
17. Nordbring-Hertz, B. and Mattiasson, B., *Nature*, 1979, **281**, 477–479.
18. Peumans, W. J. and Van Damme, E. J. M., *Plant Physiol.*, 1995, **109**, 347–352.
19. Urwin, P. E., Moller, S. G., Lilley, C. J., McPherson, M. J. and Atkinson, H. J., *Mol. Plant. Microbe Interact.*, 1997, **10**, 394–400.
20. Zuckerman, B. M., *J. Nematol.*, 1983, **15**, 173–182.
21. Borrebaeck, C. A. K., Mattiasson, B. and Nordbring-Hertz, B., *FEMS Microbiol. Lett.*, 1985, **27**, 35–39.

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