



(51) International Patent Classification: Not classified

(21) International Application Number:

PCT/IN2010/000056

(22) International Filing Date:

2 February 2010 (02.02.2010)

(25) Filing Language:

English

(26) Publication Language:

English

(71) Applicants (for all designated States except US): **UNICHEM LABORATORIES LTD.** [IN/IN]; Mahalaxmi Chambers, 22 Bhulabhai Desai Road, 400026 Mumbai, Maharashtra (IN). **KARNATAK UNIVERSITY** [IN/IN]; Dharwad, Karnatak, 580 003 (IN).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **SWAMY, Bale Murgu** [IN/IN]; c/o Department of Biochemistry, Karnatak University, 580 003 Dharwad, Karnatak (IN). **INAMDAR, Shashikala Ramchandra** [IN/IN]; c/o Department of Biochemistry, Karnatak University, 580 003 Dharwad, Karnatak (IN). **VENKAT, Hemalatha** [IN/IN]; c/o Unichem Laboratories Ltd., Biosciences R & D Centre, SID, IISc Campus, 560 012 Bangalore, Karnatak (IN). **S., Radhika** [IN/IN]; c/o Unichem Laboratories Ltd., Biosciences R & D Centre, SID, IISc Campus, 560 012 Bangalore, Karnatak (IN). **CHACHADI, Vishwanath Basavaraj** [IN/IN]; c/o Department of Biochemistry, Karnatak University, 580 003 Dharwad, Karnatak (IN). **NAGRE, Nagaraja Narayan** [IN/IN]; c/o Department of Biochemistry, Karnatak University, 580 003 Dharwad, Karnatak (IN). **GONCHIGAR, Sathisha J.** [IN/IN]; c/o Department of Biochemistry, Karnatak University, 580 003 Dharwad, Karnatak (IN). **MORYE, Vinita** [IN/IN]; c/o Unichem Laboratories Ltd., Biosciences R & D Centre, SID, IISc Campus, 560 012 Bangalore, Karnatak (IN). **RAMADOSS, Candalai Seshadri** [IN/IN]; c/o Unichem Laborat-

ories Ltd., Biosciences R & D Centre, SID, IISc Campus, 560 012 Bangalore, Karnatak (IN).

(74) Agents: **SHANKER, Archana** et al.; ANAND AND ANAND, B-41, Nizamuddin East, 110 013 New Delhi (IN).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to the identity of the inventor (Rule 4.17(i))
- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- of inventorship (Rule 4.17(iv))

Published:

- without international search report and to be republished upon receipt of that report (Rule 48.2(g))

(54) Title: RECOMBINANT LECTIN AND METHOD OF PREPARING THEREOF

(57) Abstract: The present invention provides a method for preparing a recombinant lectin expressed in a host cell such as *E.coli* or yeast, said method comprising the synthesis of a lectin gene based on amino acid sequence derived from the MALDI MS/MS of the *Sclerotium rolfii* lectin (SEQ ID.1), a soil borne phytopathogenic fungus, and its cloning in the host cell.



WO 2014/203261 A2

RECOMBINANT LECTIN AND METHOD OF PREPARING THEREOF

FIELD OF INVENTION

The invention relates to the field of carbohydrate binding proteins, and more specifically to a protein which binds specifically to particular oligosaccharides associated with particular medical significance. The invention specifically relates to lectins, more particularly to recombinant fungal lectins and method of preparation thereof.

BACKGROUND OF INVENTION

Lectins are proteins, or glycoproteins that agglutinate erythrocytes of some or all blood groups in vitro. They are important group of bioactive proteins found in most organisms. Lectins are used as tools for diagnostic and therapeutic purpose in health care areas. Lectins are also used in the purification of glycoproteins, oligosaccharide analysis and in cell selection processes. Lectins can bind reversibly with monosaccharides or the sugar moiety found in polysaccharides, glycoproteins or glycolipids.

Lectins are currently being considered for use as cancer therapeutic agents. Some lectins have been shown to bind preferentially to cancer cell membranes and cause inhibition of cell growth. There are also reports of inhibition of angiogenesis. Lectins could inhibit cell adhesion, proliferation and colony formation. Some lectins modulate the growth, through proliferation or apoptosis of premalignant and malignant cells both in vitro and in vivo. Most of these effects are mediated by specific cell surface receptors. . Because of their selective cell specificity, lectins may serve as carriers for targeted drug delivery. Some of the lectins also show anti tumor activity.

5 Several lectins have been isolated and characterized from a variety of sources. They however differ in their physico-chemical properties such as molecular size and sugar specificities. There is also characterization of a few recombinant proteins. The recombinant mushroom lectin from *Marasmius oreades* has a molecular size of 33 kDa and shows high affinity for Gal α 1, 3Gal and Gal α 1,
10 3Gal β 1, 4GlcNAc. (R.P.Kruger et al., J.Biol.Chem., 277, 15002-15005, 2002; H.C.Winter et al. J.Biol.Chem ,277, 14996-15001, 2002., I.J. Goldstein, et al. US Patent :6958321)

Peanut lectin that shows affinity to Thomsen-Friedenreich antigen has been
15 cloned (V.Sharma and A.Surolia, Gene (Amst), 148, 299-304, 1994) and subsequently some mutants of this protein with a subunit molecular mass of ~28kDa has also been expressed in *E.coli* (V.Sharma, M.Vijayan and A.Surolia, J. Biol. Chem., 271, 21200-21213,1996) with some difference in its preference for sugar specificity. A much smaller lectin of molecular mass of 11.73 kDa with
20 high binding affinity to L-fucose from *Pseudomonas aeruginosa* has also been expressed in *E.coli* as a Yellow fluorescent Protein-lectin fusion (BioTechniques, 41, 327-332, 2006).

Lectins thus have enormous potential in the area of medical assays. The instant
25 application proceeds to provide certain lectins with novel characteristics so as to be useful in the medical field.

SUMMARY OF THE INVENTION

30 The present invention provides a method for preparing a recombinant lectin expressed in a host cell such as *E.coli* or yeast. This method comprises the synthesis of a lectin gene based on amino acid sequence derived from the MALDI MS/MS of the *Sclerotium rolfsii* lectin (SEQ ID.1), a soil borne phytopathogenic fungus, and its cloning in the host cell.

35

- 5 The recombinant lectin expressed as a soluble protein is purified by ion exchange and gel filtration chromatographic techniques.

The present invention provides a recombinant lectin that is specific for blood group A1 cell and has sugar specificity towards GalNac α 1-3 (Fuc α 1-2) Gal β 1-R
10 also N-acetyl galactosamine, Tn antigen but not towards TF antigen.

Further, the present invention provides a lectin that binds to human colon cancer, ovarian cancer and leukemic cells.

- 15 The amino acid sequence SEQ ID 3 represents such lectin having all the above characteristics. The nucleic acid molecule encoding the amino acid for the protein of the above characteristics is also within the scope of the application. The DNA sequence of SEQ ID 2 represents such nucleic acid encoding the lectin having the above characteristics. The sequences also envisage any change in
20 the nucleotide or amino-acid sequences including but not limited to any substitution, deletion, addition or modification such as acetylation, nitration, pegylation, sulphonation etc. in any of the positions of the entire sequence and also to include truncations at 5' or 3' end of the sequences which do not alter the properties disclosed in the instant application.

25

In one aspect this recombinant lectin in a biotinylated form or modified with a fluorogenic chromophore can be used for the detection of cancer cells or cancer associated specific antigens.

- 30 The growth modulatory effect of the recombinant lectin can have potential application in cancer therapy

In another aspect the recombinant lectin can be used as a drug delivery agent for cancer treatment.

35

5 The application particularly discloses a DNA sequence (SEQ ID 2) coding for a 141 amino acids long lectin similar to the lectin (*S.rolfsii*), but with a few modifications was chemically synthesized and cloned into *E.coli*.

10 The method for producing said recombinant lectin has also been disclosed. The cloned lectin is expressed after induction with the inducer IPTG. The expressed protein appears in soluble and active form. The recombinant lectin is purified to essential homogeneity by resorting to anion exchange and molecular sieve chromatography. The mass spectrometric estimate of molecular weight show a value of 16.1 kDa. The calculated PI value is 4.89.

15 The recombinant lectin exhibits specificity towards blood group A1 and can be used for blood typing. The lectin binds specifically to human breast, colon and ovarian cancer tissues and is distinct from other TF antigen binding lectins. Hence the lectin has application in cancer diagnosis. The lectin exhibits substrate
20 specificity towards N-acetyl galactosamine, mucin and feutin. The lectin can be immobilized on a matrix and used for affinity purification of glycoproteins and glycoconjugates that bind to this recombinant lectin.

This recombinant lectin can be used for the detection of certain glycoproteins and
25 glycoconjugates present in biological fluids. This lectin can be used as a carrier of drugs to target certain cancer cells. The lectin may also find application as a diagnostic tool in the detection of microbial / viral pathogens.

BRIEF DESCRIPTION OF FIGURES

30

Figure 1: The Amino acid sequence of lectin protein isolated from *S. rolfsii* (SEQ ID 1)

5 Figure 2: **A DNA sequence encoding the lectin** of the instant application, SEQ ID 2. The length of the optimized sequence is 441 nucleotides which includes the 5' end Nde I (CATATG) and 3' end BamHI (GGATCC) restriction sites.

10 **Figure 3: Protein Sequence of the recombinant lectin** of the instant application. The number of amino acid coding for this lectin is 141 and is represented by SEQ ID 3. The altered/modified positions have been highlighted in the sequence.

15 **Figure 4: Multiple sequence alignment (CLUSTAL 2.0.3)** of the SEQ ID 1 and SEQ ID 3.

DETAILED DESCRIPTION OF THE INVENTION

20 The instant application provides for altered/modified lectin protein of fungal origin. The lectin purified from the soil fungus *Sclerotium rolfsii* is the basis of the invention. An altered form of the gene sequence of the native *S.rolfsii* has been cloned and subsequently expressed as a recombinant protein. The complete description in terms of experimental data is herewith provided for one representative host *E.coli*:

25 The words modify and alter have been interchangeably used in the specification to indicate any change in the nucleotide or amino-acid sequences including but not limited to any substitution, deletion, the modification to include truncations at 5' or 3' end of the sequences which may result in a protein of reduced size but
30 retaining the bioactivity. The word modification would also include chemical modification of the derived recombinant protein by resorting to pegylation, nitration, glycosylation, phosphorylation etc.

Lectin Gene construct and cloning:

35 The amino acid sequence derived from the MALDI MS/MS and x-ray crystallography data of the lectin purified from the soil fungus *Sclerotium rolfsii*

5 formed the basis for the gene construct. The gene corresponding to this sequence SEQ ID 1 was chemically synthesized by following the standard protocols. The assembled gene was first inserted into a pUC57 vector. The insert was released by digesting the plasmid with NdeI & BamHI and then recloned in to pET20b previously digested NdeI and BamHI. After ligation the
10 plasmid was used to transform *E.coli* DE3 (GOLD) host strain for expression. The recombinant clones were analyzed for release of insert after digestion with Nde I and Bam HI. The SDS-PAGE analysis of recombinant *E.coli* upon induction with IPTG showed the expected ~16kDa protein.

15 Cell growth:

A single colony of the recombinant *E.coli* was inoculated into 5ml LB-Amp allowed to grow at 37°C overnight with shaking. The overnight grown culture was inoculated into the Fermentation Media and grown at 37°C till an OD of ~2.0 was reached, the cultures were then induced with 250µM IPTG (final concentration)
20 and grown overnight at 20°C.

Cell extract preparation:

The overnight grown cultures were centrifuged at 8000rpm for 10min and the pelleted cells (4gms) were suspended in 40ml of 50mM Tris-HCl pH8.0
25 containing 1mM PMSF and 1mM EDTA. The cell suspension was sonicated for 20min using the sonicator. The cells were centrifuged at 12000rpm, 10min at 4°C. The supernatant was used for further purification. At higher growth temperatures substantial amount of the recombinant protein was found in the inclusion body fraction.

30 Purification of the protein:

DEAE-cellulose chromatography:

The supernatant (400mg of protein) in 50 mM Tris-HCl buffer pH 8.0 was loaded on 25ml DEAE (BIORAD) column equilibrated in 50mM Tris-HCl buffer pH 8.0.

35 The column was washed with 2 column volumes of buffer followed by step-wise

- 5 elution of the column with buffer b containing 75mM NaCl and 200mM NaCl. The lectin was eluted with buffer containing 300mM NaCl. This fraction contained 216 mg of protein.

PEI chromatography:

- 10 The DEAE elute (216mg) was loaded on to the NUCLEOSIL 4000-7-PEI (250 x 10mm) column, equilibrated with 50 mM Tris-acetate buffer pH 8.0. The bound protein was eluted by applying a linear gradient of NaCl from 0-100% in 15min at 2ml/min flow rate using the FPLC system and the protein eluted at 98%B (60mg). The eluted protein was dialyzed against water and repurified on another PEI
15 column preequilibrated with 20mM ammonium bicarbonate buffer pH 8.0. The bound protein was eluted using buffer gradient from 20-500mM ammonium bicarbonate pH 8.0. The eluted protein (30mg) was dialyzed against water, lyophilized and stored at -20°C.

- 20 Gel filtration chromatography: The final purification of the recombinant protein was achieved by gel filtration chromatography on Superdex G-75 equilibrated with 25 mM TBS, pH 7.2 on AKTA Prime plus purification system.

- The expressed protein and the purified fractions were analyzed on 15% SDS
25 PAGE. The purified fraction was visualized by staining with Coomassie blue. The purified protein appeared to be essentially pure and corresponded to a molecular size of about 16.kDa.

EXAMPLES

- 30 **Example 1:** Identification of neoglycans expressed on activated and transformed lymphocytes in comparison with normal lymphocytes by the recombinant lectin;

- In order to identify the neoglycoproteins expressed on lymphocytes upon
35 activation by PHA and transformed cells, the interaction studies were carried out with respective cell membrane proteins with biotinylated lectin.

5

Isolation of Cell Membrane Proteins:

Human peripheral lymphocytes were prepared from the blood collected from a healthy donor. The blood sample (20ml) collected in to glass centrifuge tube containing EDTA (12.5 mM final concentration) and the normal lymphocytes were separated by centrifugation in Ficoll-Hypaque (Pharmacia) gradient. A portion of normal lymphocytes were activated by incubating with PHA (2.5 µg/ml PHA) in RPMI-1640 medium supplemented with 10% FCS for 72 hours in CO₂ incubator. Activated cells were harvested by centrifugation and the activation was confirmed by checking for the expression of CD25 receptors.

15

Normal lymphocytes, activated lymphocytes and leukemic cell lines; Molt-4 and Jurkat were finally maintained in RPMI 1640 culture medium, supplemented with glutamine (2 mM), heat inactivated FCS (10%), penicillin (100U/ml) and streptomycin (100-ug/ml). The cultures were maintained at 37°C in humidified atmosphere (95% air and 5% CO₂). Membrane proteins from these cultured cells were isolated by using "Pierce Membrane Protein Extraction Reagent kit" method (Mem-PER, Prod #89826) and the protein contents were estimated using Bio-Rad's DC-Protein estimation Kit. Isolated membrane proteins were finally concentrated by chloroform-methanol precipitation, and the precipitated proteins were air dried and stored at 4°C till further use.

25

Electrophoresis & Western Blotting:

Polyacrylamide gel electrophoresis was performed in the presence of SDS using a minigel system (Hoefer Scientific Instruments, USA) in 10% gel for 60 mins at 120 volts. The proteins were transferred from polyacrylamide gels to Nitro Cellulose membranes in a semidry blotting equipment using transfer buffer (240mM Glycine, 25mM Tris, 0.1% SDS) containing 20% methanol at 75mA for 3hr at 4 °C. Membranes were saturated after transfer with blocking solution (3% p-BSA) and washed with TBST. Lectin binding was carried out at room temperature for 4 hours with biotin-coupled lectin at the final concentration of

35

5 20µg/ml (prepared in TBST). After thorough washing, blot was incubated with Streptavidin-HRP (1:1000 in TBST) at room temperature for 1hr and excess Streptavidin-HRP was washed with TBST. Finally the lectin-glycoproteins bands were visualized by developing with DAB chromogenic system.

10 The results of these findings show that the present recombinant lectin has the ability to bind to some of the uniquely expressed neoglycans on the leukemic cells and also activated lymphocytes.

EXAMPLE 2: Identification of Lectin binding glycoproteins of the Ovarian Cyst

15 fluid

Ovarian cyst fluid was collected aseptically from a patient admitted to SDM college of Medical Sciences and Hospital. The fluid was diluted thrice with PBS (50 mM, pH 7.2) containing 1mM PMSF and centrifuged at 10,000 rpm, for 20
20 minutes at 4 °C to obtain the clear supernatant. Clear supernatant was filtered through a membrane filter (0.25 µm, Millipore Ind. Ltd.) and dialyzed first against PBS (50 mM, pH 7.2) and subsequently with several changes of deionized water. After extensive dialysis fluid was distributed in small vials and freeze dried and stored at -20° C till further use.

25

SDS PAGE and lectin blotting

Proteins from the ovarian cyst fluids samples were separated by SDS PAGE in 10 % gels as described by Laemmli et al using Hoefer Scientific Instruments, San Francisco, USA. Electrophoresis was carried out for 70 min.1 using 110 volts.
30 Immediately after the electrophoresis fractionated protein bands were resolved were blotted onto nitro cellulose membrane using semidry blot assembly. Blotted membrane was washed with distilled water and stained with Ponceau reagent to confirm the efficiency of blotting and to mark the location of standard proteins. Finally the blot was washed with TBS containing 0.1 % Tween -20 (TBST) and
35 treated with 3 % P-BSA in TBST for overnight to prevent non-specific binding.

5 After washing with TBST, blot was incubated with biotinylated lectins (20 µg/ ml in TBST) at 37⁰ C for 4-6 hours. Excess and unbound lectin was removed by washing the membrane with TBST and then the membranes were incubated with Streptavidin-HRP (1µg/ml in TBST) for one hour. Unbound Streptavidin-HRP was removed by washing with TBST. The lectin binding glycoproteins were visualized
10 by staining for peroxidase activity using DAB system. Red cell agglutination assay;;

The results of these investigations indicted the binding of the present lectin to several glycoproteins present in the ovarian cyst fluid.

15 Hemagglutination assay:

The hemagglutinating activity of the lectin was routinely assayed by serial two fold dilution technique of Liener and Hill (J.Nutr., 49, 609-620, 1953) in microtuter plates using trypsinised human erythrocytes. The highest dilution of
20 the lectin preparation causing visible hemagglutination was regarded as titer and the protein content in the highest dilution causing visible agglutination as 1 unit of hemagglutination activity. The specific activity is expressed as units per milligram of protein.

25 Results of the hemagglutination assay show that the recombinant lectin agglutinates only the red blood cells of A1 blood group human individuals, not those of B or O group. Also the activity of agglutination by the recombinant lectin is two and half times higher than the Dolichos biflorus lectin

30 Recombinant Sequences

The gene sequences and the proteins having the following biological activity: specific binding to blood group A1 cells, sugar specificity towards GalNacα1-3 (Fucα1-2) Galβ1-R and N-acetyl galactosamine, Tn antigen, no specificity
35 towards TF antigen, substrate specificity towards N-acetyl galactosamine, mucin

5 and feutin, and specific binding to breast cancer colon cancer, ovarian cancer and leukemic cells are covered within the scope of the application. The nucleotide molecules encoding the recombinant molecules may be DNA or RNA. The proteins of the above mentioned characteristics whether obtained by deliberate or random mutations are covered within the concept of the instant
10 application.

In one of the preferred embodiments, the expressed lectin protein (SEQ ID 3) differs from the native lectin protein (SEQ ID 2) in terms of atleast one amino acid modification/substitution at positions shown in figure 4. Of these, positions 1, 34,
15 109 have amino acid substitutions: T to V, T to S and L to N respectively. There are other modifications such as Asparagine to Aspartic acid and Glutamine to Glutamic acid substitutions.

The recombinant lectin sequences can be biotinylated or attached with
20 fluorogenic chromophore by using commonly known methods to be used for the detection of cancer cells or cancer associated specific antigens. The growth modulatory effect of the recombinant lectin finds its application in cancer therapy. Further the use of recombinant lectin as a drug delivery agent for cancer treatment is envisaged in the present application.

25 While the present invention has been described with respect to certain preferred embodiments, it will be apparent to those skilled in the art that various changes and modifications may be made without departing from the scope of the invention as defined in the following claims.

30

5 **We Claim:**

1. A lectin protein comprising of an amino acid sequence of SEQ ID 1 altered /modified to have at least one of the following biological activity: specific binding to blood group A1 cells, sugar specificity towards GalNac α 1-3 (Fuc α 1-2) Gal β 1-R and N-acetyl galactosamine Tn antigen, no specificity towards TF antigen, substrate specificity towards N-acetyl galactosamine, mucin and feutin, and specific binding to breast cancer colon cancer, ovarian cancer and leukemic cells.
2. The lectin protein as claimed in claim 1 wherein said lectin protein further identifies neoglycans expressed on activated and transformed lymphocytes.
3. The lectin protein as claimed in claim 1 or 2 wherein said protein has a molecular mass of about 16.2 kDa and the calculated PI value of about 4.89.
4. The lectin protein as claimed in claim 1 or 2 wherein said alteration/modification of SEQ ID 1 is at any one of the following positions 1(Ac-T $\rightarrow$ V), 34(T $\rightarrow$ S) and 109(L $\rightarrow$ N).
5. The lectin protein as claimed in claim 1 or 2 wherein said alteration/modification of SEQ ID 1 is of following substitutions: Asn(N) $\rightarrow$ Asp(D) and Gln(Q) $\rightarrow$ Glu(E).
6. The lectin protein as claimed in any preceding claim wherein said altered/modified sequence is SEQ ID No. 3.
7. The lectin protein as claimed in any preceding claim as and when used in detection of cancer cell, cancer diagnosis or cancer therapy.

- 5 8. A nucleic acid molecule encoding a lectin protein having the following biological activities: specific binding to blood group A1 cells, sugar specificity towards GalNac α 1-3 (Fuc α 1-2) Gal β 1-R and N-acetyl galactosamine Tn antigen, no specificity towards TF antigen, substrate specificity towards N-acetyl galactosamine, mucin and feutin, and specific binding to breast cancer
10 colon cancer, ovarian cancer and leukemic cells.
9. The nucleic acid molecule as claimed in claim 8 wherein said nucleic acid molecule is DNA or RNA.
10. The nucleic acid molecule as claimed in claim 8 wherein said lectin protein is altered/modified *Sclerotium rolfsii* lectin protein of the amino acid sequence of
15 SEQ ID 1.
11. The nucleic acid molecule as claimed in claim 8 wherein said lectin protein comprises the amino acid sequence of SEQ ID 3.
12. The nucleic acid molecule as claimed in claim 8 or 9 wherein said nucleic acid molecule is a DNA sequence comprising sequence SEQ ID 2.
- 20 13. A recombinant vector comprising operatively linked in the 5' to 3' direction: a promoter which functions in a host cell; a structural nucleic acid sequence as claimed in claims 8-12, wherein said nucleic acid sequence encodes a lectin protein; and a termination signal.
14. A recombinant vector as claimed in claim 13 wherein said recombinant
25 vector is capable of being replicated, transcribed, translated and expressed in a unicellular organism.
15. A transformed host cell containing the vector as claimed in claim 13.
16. The transformed host cell as claimed in claim 8 wherein said host cell is *Escherichia coli* bacterium or yeast cell.
- 30

- 5 17. A process for producing a recombinant *Sclerotium rolfsii* lectin protein
comprising:
- culturing a host cell containing the recombinant vector of claim 13 coding
for the lectin protein;
 - expressing the recombinant lectin protein
- 10 • isolating said lectin protein from the culture.

SHEET 1/4**SEQ ID 1:**

Ac-T-Y-K-I-T-V-R-V-Y-Q-T-N-P-N-A-F-F-H-P-V-E-K-T-V-W-K-Y-A-N-G-G-T-W-T-I-T-D-D-Q-H-V-L-T-M-G-G-S-G-T-S-G-T-L-R-F-H-A-D-N-G-E-S-F-T-A-T-F-G-V-H-N-Y-K-R-W-C-D-I-V-T-N-L-A-A-D-E-T-G-M-V-I-N-Q-Q-Y-Y-S-Q-K-N-R-E-E-A-R-E-R-Q-L-S-N-Y-E-V-K-N-A-K-G-R-N-F-E-I-V-Y-T-E-A-E-G-N-D-L-H-A-N-L-I-I-G-COOH

FIGURE 1

SHEET 2/4**SEQ ID 2**

CAT ATG GTG TAT AAA ATT ACC GTT CGC GTG TAT CAG ACC
AAT CCG GAT GCG TTT TTC CAT CCG GTG GAA AAA ACC GTG
TGG AAA TAT GCG GAT GGC GGT ACC TGG AGC ATT ACC GAT
GAT CAG CAC GTT CTG ACC ATG GGC GGT AGC GGC ACC AGC
GGC ACC CTG CGC TTT CAT GCG GAT AAT GGT GAA AGC TTT
ACC GCG ACC TTT GGC GTG CAT GAT TAT AAA CGT TGG TGC
GAT ATT GTG ACC GAT CTG GCG GCG GAT GAA ACC GGT ATG
GTG ATT AAT CAG GAA TAT TAT AGC GAA AAA GAT CGC GAA
GAA GCG CGC GAA CGT CAG AAT AGC AAC TAT GAA GTG AAA
GAT GCC AAA GGT CGT AAC TTT GAA ATT GTG TAT ACC GAA
GCC GAA GGT AAT GAT CTG CAT GCG GAT CTG ATT ATT GGC
TAA TAA GGA TCC

FIGURE 2

SHEET 3/4**SEQ ID 3:**

YYKITVRVYQTNPDAFFHPVEKTVWKYADDGGTWSITDDQHVLTMGGSGTSGTL
RFHADNG 60

ESFTATFGVHDYKRWCDIVTDLAADETGMVINQEYYSEKDREEARERQNSNE
VKDAKGR 120

NFEIVYTEAEGNDLHADLIIG 141

FIGURE 3

FIGURE 4