

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
2 December 2004 (02.12.2004)

PCT

(10) International Publication Number
WO 2004/104193 A2

(51) International Patent Classification⁷: **C12N 9/20**,
15/10, A21D 8/04, A61K 38/43

(74) Agents: **WOODS, Geoffrey, Corlett** et al.; J.A. Kemp &
Co., 14 South Square, Gray's Inn, London WC1R 5JJ (GB).

(21) International Application Number:
PCT/GB2004/002223

(22) International Filing Date: 24 May 2004 (24.05.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0312037.5 24 May 2003 (24.05.2003) GB

(71) Applicant (for all designated States except US): **F2G LTD**
[GB/GB]; Lankro Way, Eccles, Manchester, Greater Man-
chester, M30 0BH (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **BIRCH, Michael**
[GB/GB]; F2G Limited, Lankro Way, Eccles, Manchester,
Greater Manchester M30 0BH (GB). **LAVENS, San-
dra, Elizabeth** [GB/GB]; F2G Limited, Lankro Way,
Eccles, Manchester, Greater Manchester M30 0BH (GB).
RYCROFT, Catherine, Elaine [GB/GB]; F2G Limited,
Lankro Way, Eccles, Manchester, Greater Manchester
M30 0BH (GB). **TUCKWELL, Daniel, Scott** [GB/GB];
F2G Limited, Lankro Way, Eccles, Manchester, Greater
Manchester M30 0BH (GB).

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, 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, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI,
SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished
upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: PHOSPHOLIPASE C

(57) Abstract: The invention provides a polynucleotide comprising a PLC gene isolated from an organism independently selected from a group of genera consisting of *Aspergillus*; *Blumeria*; *Candida*; *Cryptococcus*; *Encephalitozoon*; *Fusarium*; *Leptosphaeria*; *Magnaporthe*; *Phytophthora*; *Plasmopara*; *Pneumocystis*; *Pyricularia*; *Pythium*; *Puccinia*; *Rhizoctonia*; *Trichophyton*; and *Ustilago*. The invention further provides a PLC polypeptide isolated from an organism independently selected from a group of genera consisting of *Aspergillus*; *Blumeria*; *Candida*; *Cryptococcus*; *Encephalitozoon*; *Fusarium*; *Leptosphaeria*; *Magnaporthe*; *Phytophthora*; *Plasmopara*; *Pneumocystis*; *Pyricularia*; *Pythium*; *Puccinia*; *Rhizoctonia*; *Trichophyton*; and *Ustilago*.



WO 2004/104193 A2

PHOSPHOLIPASE C

The present invention relates to phospholipase C (PLC) enzymes. In particular, the
5 invention relates to fungal extracellular PLC genes, and encoded proteins thereof,
including variants, derivatives and analogues thereof, and their uses in diagnosis and
therapy.

Phospholipases are a broad group of enzymes that break down phospholipids, a
ubiquitous class of molecule found in both eukaryotes and prokaryotes.
10 Phospholipids are important structural molecules, forming the lipid bilayers of
membranes. Phospholipids and their break down products also play a pivotal role in
many signalling pathways.

Phospholipids are amphipathic molecules and are generally assembled into
bilayers or micelles with very little free phospholipid present within cells. They are
15 defined by their polar head group, the most common of which is phosphatidylcholine
(PC). Other common phospholipid groups are phosphatidylethanolamine (PE),
phosphatidylserine (PS), phosphatidylinositol (PI) and phosphatidylglycerol (PG).
Within these broad classes of phospholipids, variation in fatty acid chain length and
the degree of saturation are also observed. These factors play an important role in
20 determining the substrate specificity of individual phospholipases, as does the
manner in which the phospholipid is presented, i.e., whether it occurs as a free
molecule, in bilayers or multi-lamellae complexes.

Two distinct classes of phospholipases are known, phosphodiesterases and acyl
hydrolases. Phosphodiesterases, such as phospholipases C and D, hydrolyse the
25 phosphate bond, while acyl hydrolases, such as phospholipases A1, A2 and B and
lysophospholipase, cleave the fatty acid substituents from the glycerophosphatidyl
backbone. Phospholipase C enzymes (PLC; EC. 3.1.4.3) hydrolyse the
phosphodiester bond between the phosphate oxygen and the glycerol carbon,
liberating a diacylglycerol (DAG) molecule and headgroup-phosphate complex.
30 PLCs may have wide substrate specificity acting on a variety of phospholipid classes
including sphingomyelin (which is based on the amino alcohol sphingosine, rather

than glycerol), although activity is often optimal against a specific class of phospholipid.

PLCs are subdivided into intracellular and extracellular PLCs. Intracellular PLCs act exclusively within a cell, controlling phospholipid turnover and have important signalling roles, activating protein kinase C and the arachidonic acid cascade through the formation of DAG. These PLCs are found in both prokaryotes and eukaryotes and are exemplified by the mammalian phosphoinositol specific PLC (PI-PLC). Extracellular PLCs are secreted phospholipases and have been found in bacteria (Titball, 1993, Microbiol. Rev. 57, 347-366). A number of classes of bacterial extracellular PLCs have been identified, such as PLC-H and PLC-N of *Pseudomonas aeruginosa*, and the *Clostridium perfringens* α toxin (Titball and Sanger, 1998, Soc. Appl. Bacteriol. Symp. 27, 127137s). Significantly, there is no apparent sequence relationship between the intracellular and extracellular PLCs, and little or none between the extracellular PLC classes. Previously, no extracellular PLC genes had been isolated from fungi.

Damage to cells and tissues is perhaps the most widely recognised virulence effect of bacterial PLCs. Several PLCs have been shown to be haemolytic to erythrocytes and to have general cytolytic activity. *Clostridium perfringens* α toxin is the most potent cytolytic PLC known and has been shown to play an essential role in the pathogenesis of gas gangrene, directly hydrolysing membrane phospholipids (El-Sayed et al., 1985, Biophys Acta 837:325-335). α toxin has also been shown to induce the expression of human cytokines, promote inflammatory responses and to trigger the arachidonic acid cascade (Titball, 1993; Sanger, 1997, Trends Microbiol. 5, 156-161).

Fungal PLCs could play an important role in the progression and pathogenesis of fungal infections. The invasion of host cells by fungi involves the penetration and damage of cell membranes by physical or enzymatic means. Since phospholipids are major components of the cell membrane, the production of extracellular phospholipases by an invading organism may be a significant factor in pathogenesis, as has been demonstrated for bacterial infections. In the case of pulmonary pathogens, the presence of extracellular PLCs may be of particular significance since the lung and the alveoli are coated with lung surfactant consisting of up to 80%

phospholipid. Breakdown of this lung surfactant, by extracellular PLCs could lead to a breakdown in localised oxygen tensions.

Phospholipases also have many industrial applications, including the modification of phospholipid emulsifiers. An example of a phospholipid emulsifier is lecithin, which is a mixture of both polar and neutral lipids in which the content of polar lipids is at least 60%. Phospholipid emulsifiers have many food and non-food applications, for example egg-lecithin is used as an emulsifier in for example dairy products, specifically mayonnaise, dressings, pastry, etc., soya lecithin for example, is for example used as an emulsifier in (low calorie) sauces, bread, margarine, cosmetics etc, other lecithins are used in for example chocolates, calf feed. Modification of phospholipid emulsifiers by phospholipases may cause an increased emulsification of the oil/water mixture. Modification of phospholipid emulsifiers by phospholipases may increase the stability of the emulsions resulting from the addition of the modified phospholipid emulsifiers for a wider or different pH and/or temperature range. Modification of phospholipid emulsifiers by phospholipases may increase the stability of the emulsions, resulting from the addition of modified phospholipid emulsifiers, in the presence of Ca^{2+} or Mg^{2+} ions.

Another example of industrial application of phospholipases is that they can be used for the degumming of vegetable oils in the processing of these oils. In a typical degumming process, lecithins are removed from vegetable oils, for example soy oils, rapeseed (canola) oils, linseed oils, sunflower oils, to increase among others the stability of the vegetable oil, by washing the oil phase with water, wherein mixing of the water and oil under high shear conditions forces the bulk of the lecithins into the aqueous phase, which is subsequently removed in a separator. In this so-called water degumming phase, only the rapidly hydratable phospholipids are readily removed, for example phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine. The non- hydratable phospholipids/phosphatides, mostly the phospholipids, which consist of up to 50% of magnesium and/or calcium salts cannot readily be removed in the water degumming step. Exposure of the non-hydratable phospholipids/phosphatides to phospholipase can make these phospholipids more soluble in water and therefore easier to extract in a water degumming phase. Another example of industrial application of phospholipases is

that they are used to remove the precipitate that occurs during the saccharification (with the aid of α -amylase and glucoamylase) of wheat gluten or wheat starch to produce glucose syrups. The removal of the precipitate considerably speeds up the subsequent filtration of the resulting glucose syrups. The above-mentioned industrial applications of the phospholipase enzyme are only a few examples and this listing is not meant to be restrictive.

Yet another example of an industrial application of phospholipases in food is that phospholipases are particularly useful in baking applications to improve dough or baked product quality. Wheat flour contains approximately 2.2-2.9% lipids. The flour lipids can be divided into starch lipids (0.8-0.9%) and non-starch lipids (1.4-2.0%). Whereas the starch lipids consist mainly of polar lysophospholipids, the non-starch lipids consist of about 40% neutral triglycerides and 40% polar phospho- and glycolipids. For optimisation of the flour lipids fraction it is possible to hydrolyse the phospholipids in situ in the dough by adding phospholipase A.

As used herein, the terms "PLC", "extracellular PLC", "extracellular PLC polypeptide" and "extracellular PLC protein" are defined as nucleic acids or proteins which: (a) match the PFAM matrix PF04185 with an E-value of <0.00001 (<http://www.sanger.ac.uk/Software/Pfam/search.shtml>); and/or (b) match the *Pseudomonas aeruginosa* PLC protein (PHLC_PSEAE) or DNA (M13047) on BLAST with E-values of <0.00001 ; and/or (c) have the enzyme activity defined by EC number 3.1.4.3, noting that the substrate may not be defined as, or restricted to, phosphatidylcholine, but may include other phospholipids or sphingomyelin (<http://www.chem.qmul.ac.uk/iubmb/enzyme/EC3/1/4/3.html>); and/or (d) show enzymic activity that cleaves a phospholipid or sphingomyelin between the phosphate oxygen and the glycerol/ceramide carbon. PLCs can thus be identified either by sequence similarity to a known member of the family or by enzymic activity, since only a few of the available sequences relate to functionally characterised molecules (as is the case with most proteins). Extracellular PLCs identified on the basis of sequence similarity are validated by alignment of the sequence with known extracellular PLCs.

According to a first aspect of the present invention, there is provided an isolated polynucleotide comprising a fungal PLC gene. The PLC gene may

comprise a PLC polynucleotide that comprises:

(i) a nucleotide sequence independently selected from at least one of SEQ ID NOS: 1, 2, 4, 5, 7, 8, 10, 11, 13 or 15, or complement thereof; or

(ii) a nucleotide sequence which has sequence identity with (i); or

5 (iii) a nucleotide sequence which is a fragment of (i) or (ii);

wherein (ii) or (iii) optionally encode a polypeptide which has phospholipase activity.

According to a second aspect of the present invention, there is provided an isolated fungal PLC polypeptide. The PLC polypeptide may comprise:

(i) an amino acid sequence independently selected from at least one of SEQ ID NOS:

10 3, 6, 9, 12, 14 or 16; or

(ii) an amino acid sequence which has sequence identity with (i); or

(iii) an amino acid sequence which is a fragment of (i) or (ii);

wherein (ii) or (iii) optionally have phospholipase activity.

Surprisingly, the inventors have isolated three extracellular PLCs genes
15 (arbitrarily named PLC-A, PLC-B and PLC-C) in *A. fumigatus*, and also the corresponding polypeptides, i.e. the PLC proteins, which each of these three genes encode. Furthermore, the inventors have isolated PLC genes from *Aspergillus nidulans*, *Aspergillus niger* and *Magnaporthe grisea*.

Preferably, the isolated polynucleotide comprises a PLC gene. Preferably, the
20 isolated polypeptide comprises a PLC. Preferably, the PLC is an extracellular PLC, preferably when functional. The polynucleotide may comprise native, synthetic or recombinant polynucleotide, and the polypeptide may comprise native, synthetic or recombinant polypeptide. The polynucleotide or polypeptide may comprise combinations of native, synthetic or recombinant polynucleotide or polypeptide,
25 respectively. The polynucleotides and polypeptides of the invention may have a sequence which is the same as, or different from, naturally occurring PLC polynucleotides and polypeptides.

It is to be understood that the term "isolated from" may be read as "of" herein. Therefore references to polynucleotides and polypeptides being "isolated from" a
30 particular organism include polynucleotides and polypeptides which were prepared by means other than obtaining them from the organism, such as synthetically or recombinantly.

Preferably, the isolated polynucleotide, and preferably the isolated polypeptide, is isolated from a filamentous fungus, more preferably an Ascomycete.

Preferably, the isolated polynucleotide, and preferably the isolated polypeptide, is isolated from an organism independently selected from a group of genera
5 consisting of *Aspergillus*; *Blumeria*; *Candida*; *Cryptococcus*; *Encephalitozoon*; *Fusarium*; *Leptosphaeria*; *Magnaporthe*; *Phytophthora*; *Plasmopara*; *Pneumocystis*; *Pyricularia*; *Pythium*; *Puccinia*; *Rhizoctonia*; *Trichophyton*; and *Ustilago*.

Preferably, the isolated polynucleotide, and preferably the isolated polypeptide, is isolated from an organism independently selected from a group of genera
10 consisting of *Aspergillus* and *Magnaporthe*.

Preferably, the isolated polynucleotide, and preferably the isolated polypeptide, is isolated from an organism independently selected from a group of species consisting of *Aspergillus flavus*; *Aspergillus fumigatus*; *Aspergillus nidulans*; *Aspergillus niger*; *Aspergillus parasiticus*; *Aspergillus terreus*; *Blumeria graminis*;
15 *Candida albicans*; *Candida cruzei*; *Candida glabrata*; *Candida parapsilosis*; *Candida tropicalis*; *Cryptococcus neoformans*; *Encephalitozoon cuniculi*; *Fusarium solani*; *Leptosphaeria nodorum*; *Magnaporthe grisea*; *Phytophthora capsici*; *Phytophthora infestans*; *Plasmopara viticola*; *Pneumocystis jiroveci*; *Puccinia coronata*; *Puccinia graminis*; *Pyricularia oryzae*; *Pythium ultimum*; *Rhizoctonia solani*; *Trichophyton interdigitale*; *Trichophyton rubrum*; and *Ustilago maydis*.
20

Preferably, the isolated polynucleotide, and preferably the isolated polypeptide, is isolated from an organism independently selected from a group of species consisting of *Aspergillus fumigatus*; *Aspergillus nidulans*; *Aspergillus niger*; and *Magnaporthe grisea*.

25 The polynucleotide, and preferably the polypeptide, may be isolated from *Aspergillus*, preferably from *Aspergillus fumigatus*, and more preferably, from *A. fumigatus* AF293. The polynucleotide, and preferably the polypeptide may be isolated from *Aspergillus nidulans*, or *Aspergillus niger*. The polynucleotide, and preferably the polypeptide may be isolated from *Magnaporthe*, and more preferably,
30 from *Magnaporthe grisea*.

Bioinformatics analysis was carried out to identify functionally important regions within the fungal PLCs. The PFAM phosphoesterase profile for PLCs

(PF04185; <http://www.sanger.ac.uk/Software/Pfam/>) also detects acid phosphatases, indicating a functional similarity between these protein families. Since acid phosphatases typically show only 15-20% identity to PLCs at the amino acid level (i.e, they are not closely related), conserved or similar regions of sequence are likely to be important for basic functional properties. Therefore, a multiple alignment of fungal PLCs, plant PLCs, bacterial PLCs and related acid phosphatases was generated (see Figure 1 and Example 4 hereinafter). From this it was possible to identify five regions or motifs (Boxes II, III, V, VI, and X) that are conserved between all PLCs and acid phosphatases and are therefore predicted to play an important role in PLC function.

Variants of the above mentioned polynucleotides and polypeptides are also provided, and are discussed below.

In a first embodiment, *A. fumigatus* PLC-A, the isolated polypeptide may comprise a region or motif independently selected from at least one of the following:-

- (i) a region comprising an amino acid sequence
- (ii) substantially as set out as row PLC-A in box II as shown in Figure 1, or a variant thereof;
- (iii) a region comprising an amino acid sequence substantially as set out as row PLC-A in box III as shown in Figure 1, or a variant thereof;
- (iv) a region comprising an amino acid sequence substantially as set out as row PLC-A in box V as shown in Figure 1, or a variant thereof;
- (v) a region comprising an amino acid sequence substantially as set out as row PLC-A in box VI as shown in Figure 1, or a variant thereof; and
- (vi) a region comprising an amino acid sequence substantially as set out as row PLC-A in box X as shown in Figure 1, or a variant thereof.

At least one region or motif may be functional.

In a second embodiment, *A. fumigatus* PLC-B, the isolated polypeptide may comprise a region or motif independently selected from at least one of the following:-

- (i) a region comprising an amino acid sequence substantially as set out as row PLC-B in box II as shown in Figure 1, or a variant thereof;
- (ii) a region comprising an amino acid sequence substantially as set out as row PLC-B in box III as shown in Figure 1, or a variant thereof;

- (iii) a region comprising an amino acid sequence substantially as set out as row PLC-B in box V as shown in Figure 1, or a variant thereof;
- (iv) a region comprising an amino acid sequence substantially as set out as row PLC-B in box VI as shown in Figure 1, or a variant thereof; and
- 5 (v) a region comprising an amino acid sequence substantially as set out as row PLC-B in box X as shown in Figure 1, or a variant thereof.

In a third embodiment, *A. fumigatus* PLC-C, the isolated polypeptide may comprise a region or motif independently selected from at least one of the following:-

- 10 (i) a region comprising an amino acid sequence substantially as set out as row PLC-C in box II as shown in Figure 1, or a variant thereof;
- (ii) a region comprising an amino acid sequence substantially as set out as row PLC-C in box III as shown in Figure 1, or a variant thereof;
- (iii) a region comprising an amino acid sequence substantially as set out as row PLC-C in box V as shown in Figure 1, or a variant thereof;
- 15 (iv) a region comprising an amino acid sequence substantially as set out as row PLC-C in box VI as shown in Figure 1, or a variant thereof; and
- (v) a region comprising an amino acid sequence substantially as set out as row PLC-C in box X as shown in Figure 1, or a variant thereof.

In a fourth embodiment, *A. nidulans* PLC, the isolated polypeptide may comprise
20 a region or motif comprising an amino acid sequence substantially as set out as row Anid1 in box X as shown in Figure 1, or a variant thereof.

In a fifth embodiment, *A. niger* PLC, the isolated polypeptide may comprise a region or motif independently selected from at least one of the following:-

- 25 (i) a region comprising an amino acid sequence substantially as set out as row Anig1 in box VI as shown in Figure 1, or a variant thereof; and
- (i) a region comprising an amino acid sequence substantially as set out as row Anig1 in box X as shown in Figure 1, or a variant thereof.

In a sixth embodiment, *M. grisea* PLC, the isolated polypeptide may comprise a
30 region or motif comprising an amino acid sequence substantially as set out as row Mpor1 in box II as shown in Figure 1, or a variant thereof.

Within, or closely adjacent to, the putative functional regions identified above,

fungus-specific residues have been identified, which are conserved between most or all fungal PLCs, and which are absent from other PLCs. These residues, at positions 236, 265, 279, 365, 368, 369, 535 and 537, represent sites at which the putative active-site regions identified by homology between PLCs and acid phosphatases may acquire fungus-specific function (as shown in Figure 1).

The isolated polypeptide may comprise an amino acid sequence independently selected from at least one of the following:-

- (i) a residue shown at position 236 of rows PLC-A/PLC-B/PLC-C of box II as shown in Figure 1, or a variant thereof;
- 10 (ii) a residue shown at position 265 and/or 279 of rows PLC-A/PLC-B/PLC-C of box III as shown in Figure 1, or a variant thereof;
- (iii) a residue shown at position 365 and/or position 368 and/or position 369 of rows PLC-A/PLC-B/PLC-C of, or substantially adjacent to, box V as shown in Figure 1, or a variant thereof; and
- 15 (iv) a residue shown at position 535 and/or position 537 of rows PLC-A/PLC-B/PLC-C/Anid1/Anig1 /Mpor1 adjacent to box X as shown in Figure 1, or a variant thereof.

Analysis of the multiple alignment given in Figure 1 identified regions that were conserved between bacterial, plant and fungal PLCs, but not acid phosphatases (Boxes I, VII, and IX, as shown in Figure 1). This conservation suggests that these regions are important for the specific function of PLCs, such as binding of the lipid moiety.

The isolated polypeptide may comprise an amino acid sequence independently selected from at least one of the following:-

- 25 (i) a PLC-specificity region shown in rows PLC-A/PLC-B/PLC-C/Mpor1 of box I as shown in Figure 1, or a variant thereof;
- (ii) a PLC-specificity region shown in rows PLC-A/PLC-B/PLC-C/Anig1 of box VII as shown in Figure 1, or a variant thereof; and
- (iii) a PLC-specificity region shown in rows PLC-A/PLC-B/PLC-C/Anig1 of box IX as shown in Figure 1, or a variant thereof.
- 30

Analysis of the multiple alignment shown in Figure 1 identifies regions and amino acids that vary between the kingdom groups within the alignment but which can be

used to identify fungus-specific sequences which are good indicators of a fungal PLC (positions 98, 145, 147, 150, 320, 321, 326, 327, 345, 455 and 558; Boxes IV and VIII; as shown in Figure 1).

The isolated polypeptide may comprise an amino acid sequence independently
5 selected from at least one of the following:-

- (i) a fungal PLC-specific amino acid shown at position 98 and/or 145 and/or position 147 and/or position 150 and/or position 320 and/or position 321 and/or position 326 and/or 327 and/or 345 and/or 455 and/or position 558 of rows PLC-A/PLC-B/PLC-C/Anid1/Anig1/Mpor1 as shown in Figure 1,
10 or a variant thereof;
- (ii) a fungal PLC-specific region shown as box IV of rows PLC-A/PLC-B/PLCC as shown in Figure 1, or a variant thereof; and
- (iii) a fungal PLC-specific region shown as box VIII of rows PLC-A/PLC-B/PLC-C/Anig1 as shown in Figure 1, or a variant thereof.

15 Typically a region, motif or residue is present in substantially the same location as the equivalent location shown in Figure 1 or Table II. The equivalent location can be deduced, for example, using any suitable algorithm mentioned herein. In one embodiment the protein or polynucleotide also comprises sequence flanking the region, motif or residue as shown in Figures 1 or Table II such as sequences of length
20 at least 10, 20 or 30 amino acids/nucleotides flanking the N terminal side and/or C terminal side, or 5' and/or 3' side, of the region, motif or residue; or sequence which has percentage identity with the flanking sequence.

Table I. PLC sequences claimed and their relationship to sequences given in the sequence listing.

	gDNA/EST ¹	cDNA/mRNA	Protein
PLC-A	SEQ ID No. 1: 1658-2959	SEQ ID No. 2	SEQ ID. No. 3
PLC-B	SEQ ID No. 4: 1313-1873, 1924-2733	SEQ ID No. 5	SEQ ID No. 6
PLC-C	SEQ ID No. 7: 948-1526, 1573-2376	SEQ ID No. 8	SEQ ID No. 9
<i>Aspergillus</i> <i>nidulans</i> EST	SEQ ID No. 10: 141-315	SEQ ID No. 11	SEQ ID No. 12
<i>Aspergillus niger</i> EST	SEQ ID No. 13: 1-404	SEQ ID No. 13 1-404	SEQ ID No. 14
<i>Magnaporthe</i> <i>grisea</i> EST	SEQ ID No. 15: 21-517	SEQ ID No. 15: 21-517	SEQ ID No. 16

- 5 ⁽¹⁾Numbers after SEQ ID Nos. correspond to bases of genomic DNA encoding the protein.

The isolated polynucleotide may comprise DNA, preferably genomic DNA. Preferably, in a first embodiment, *A. fumigatus* PLC-A, the isolated polynucleotide comprises substantially the sequence as shown by bases 1658-2959 of SEQ ID
10 No.1, or a complement, or a variant thereof.

Preferably, in a second embodiment, *A. fumigatus* PLC-B, the isolated polynucleotide comprises substantially the sequence as shown by bases 1313-2733 of SEQ ID No.4, or a complement, or a variant thereof. Preferably, the isolated polynucleotide comprises a first exon as shown by bases 1313-1873 of SEQ ID No.4,
15 and preferably, a second exon as shown by bases 1924-2733 of SEQ ID No.4.

Preferably, in a third embodiment, *A. fumigatus* PLC-C, the isolated polynucleotide comprises substantially the sequence as shown by bases 948-2376 of SEQ ID No.7, or a complement, or a variant thereof. Preferably, the isolated polynucleotide comprises a first exon as shown by bases 948-1526 of SEQ ID No.7,
20 and preferably, a second exon as shown by bases 1576-2376 of SEQ ID No.7.

Preferably, in a fourth embodiment, *A. nidulans* PLC, the isolated

polynucleotide comprises substantially the sequence as shown by bases 141-315 of SEQ ID No.10, or a complement, or a variant thereof.

Preferably, in a fifth embodiment, *A. niger* PLC, the isolated polynucleotide comprises substantially the sequence as shown by bases 1-404 of SEQ ID No.13, or a
5 complement, or a variant thereof.

Preferably, in a sixth embodiment, *M. grisea* PLC, the isolated polynucleotide comprises substantially the sequence as shown by bases 21-517 of SEQ ID No.15, or a complement, or a variant thereof.

Preferably, the isolated polynucleotide encodes a PLC polypeptide which, in a
10 first embodiment, *A. fumigatus* PLC-A, preferably, comprises substantially the amino acid sequence as shown as SEQ ID No.3, or a variant thereof. In a second embodiment, *A. fumigatus* PLC-B, the polynucleotide encodes a PLC polypeptide which encodes a PLC polypeptide which, preferably comprises substantially the amino acid sequence as shown as SEQ ID No.6, or a variant thereof. In a third
15 embodiment, *A. fumigatus* PLC-C, the polynucleotide encodes a PLC polypeptide which, preferably comprises substantially the amino acid sequence as shown as SEQ ID No.9, or a variant thereof.

Preferably, in a fourth embodiment, *A. nidulans*, the polynucleotide encodes a PLC polypeptide which preferably comprises substantially the amino acid sequence
20 as shown as SEQ ID No.12, or a variant thereof. Preferably, in a fifth embodiment, *A. niger* PLC, the polynucleotide encodes a PLC polypeptide, which preferably comprises substantially the amino acid sequence as shown as SEQ ID No.14, or a variant thereof. Preferably, in a sixth embodiment, *M. grisea* PLC, the polynucleotide encodes a PLC polypeptide which, preferably comprises substantially the amino acid
25 sequence as shown as SEQ ID No.16, or a variant thereof.

The isolated polynucleotide may comprise RNA, preferably mRNA which is, preferably spliced mRNA. Preferably, in a first embodiment, *A. fumigatus* PLC-A, the isolated polynucleotide comprises substantially the sequence shown as SEQ ID No.2, or a complement, or a variant thereof. Preferably, in a second embodiment, *A.*
30 *fumigatus* PLC-B, the isolated polynucleotide comprises substantially the sequence shown as SEQ ID No.5, or a complement, or a variant thereof. Preferably, in a third embodiment, *A. fumigatus* PLC-C, the isolated polynucleotide comprises

substantially the sequence shown as SEQ ID No.8, or a complement, or a variant thereof.

Preferably, in a fourth embodiment, *A. nidulans* PLC, the isolated polynucleotide comprises substantially the sequence shown as SEQ ID No.11, or a complement, or a variant thereof. Preferably, in a fifth embodiment, *A. niger* PLC, the isolated polynucleotide comprises substantially the sequence shown as SEQ ID No.13, or a complement, or a variant thereof. Preferably, in a sixth embodiment, *M. grisea* PLC, the isolated polynucleotide comprises substantially the sequence shown as SEQ ID No.15, or a complement, or a variant thereof.

10 Preferably, in a first embodiment, *A. fumigatus* PLC-A, the polypeptide comprises substantially the sequence as shown as SEQ ID No.3, or a variant thereof. Preferably, in a second embodiment, *A. fumigatus* PLC-B, the polypeptide comprises substantially the sequence as shown as SEQ ID No.6, or a variant thereof. Preferably, in a third embodiment, *A. fumigatus* PLC-C, the polypeptide comprises substantially the sequence as shown as SEQ ID No.9, or a variant thereof. Preferably, in a fourth embodiment, *A. nidulans* PLC, the polypeptide comprises substantially the sequence as shown as SEQ ID No.12, or a variant thereof. Preferably, in a fifth embodiment, *A. niger* PLC, the polypeptide comprises substantially the sequence as shown as SEQ ID No.14, or a variant thereof. Preferably, in a fourth embodiment, *M. grisea* PLC, the polypeptide comprises substantially the sequence as shown as SEQ ID No.16, or a variant thereof.

Preferably, in a first embodiment, *A. fumigatus* PLC-A, the isolated polypeptide is encoded by the sequence shown as bases 1658-2959 of SEQ ID No.1 or a complement, or a variant thereof.

25 Preferably, in a second embodiment, *A. fumigatus* PLC-B, the isolated polypeptide is encoded by exon sequences of the polynucleotide shown in SEQ ID No.4, preferably bases 1313-1873, and preferably bases 1924-2733, or a complement, or a variant thereof. Preferably, in a third embodiment, *A. fumigatus* PLC-C, the polypeptide is encoded by the exon sequences of the polynucleotide shown in SEQ ID No.7, preferably bases 948-1526, and preferably bases 1573-2376, or a complement, or a variant thereof.

Preferably, in a fourth embodiment, *A. nidulans* PLC, the isolated polypeptide

is encoded by the sequence shown as bases 141-315 of SEQ ID No.10 or a complement, or a variant thereof.

Preferably, in a fifth embodiment, *A. niger* PLC, the isolated polypeptide is encoded by the sequence shown as bases 1-404 of SEQ ID No.13 or a complement,
5 or a variant thereof.

Preferably, in a sixth embodiment, *M. grisea* PLC, the isolated polypeptide is encoded by the sequence shown as bases 21-517 of SEQ ID No.15 or a complement, or a variant thereof.

According to a third aspect of the present invention, there is provided an
10 isolated polynucleotide comprising substantially a nucleotide sequence independently selected from at least one sequence referred to in Table II, or a complement, or a variant thereof.

Table II. Regions and domains of the PLC polynucleotide

	PLC-A		PLC-B		PLC-C	
	gDNA SEQ ID No. 1	cDNA SEQ ID No. 2	gDNA SEQ ID No. 4	cDNA SEQ ID No. 5	gDNA SEQ ID No. 7	cDNA SEQ ID No. 8
Box I	1751-1795	94-138	1430-1474	118-162	1086-1130	139-183
Box II	2087-2239	421-582	1775-1986	463-624	1428-1635	481-642
Box III	2273-2323	616-666	2020-2070	658-708	1669-1719	676-726
Box IV	2333-2374	676-717	2083-2124	721-762	1732-1773	739-780
Box V	2438-2467	781-810	2185-2214	823-852	1831-1860	838-867
Box VI	2429-2614	835-957	2245-2367	883-1005	1891-2013	898-1020
Box VII	2615-2632	958-975	2368-2385	1006-1023	2014-2031	1021-1038
Box VIII	2666-2713	1009-1056	2416-2463	1054-1101	2062-2109	1069-1116
Box IX	2717-2779	1060-1122	2467-2529	1105-1167	2113-2175	1120-1182
Box X	2804-2854	1147-1197	2563-2613	1201-1251	2212-2262	1219-1269
Position 73	1805-1807	148-150	1484-1486	172-174	1140-1142	193-195
Position 99	1838-1840	181-183	1514-1516	202-204	1170-1172	223-225
Position 145	1970-1972	313-315	1649-1651	337-339	1305-1307	358-360
Position 147	1976-1978	319-321	1655-1657	343-345	1311-1313	364-366
Position 150	1985-1987	328-330	1664-1666	352-354	1320-1322	373-375
Position 236	2225-2227	568-570	1972-1974	610-612	1621-1623	628-630

Position 265	2273-2275	616-618	2020-2022	658-660	1669-1671	676-678
Position 279	2315-2317	658-660	2062-2064	700-702	1711-1713	718-720
Position 320, 321	2345-2350	688-693	2095-2100	733-738	1744-1749	751-756
Position 326, 327	2363-2368	706-711	2113-2118	751-756	1762-1767	769-774
Position 345	2402-2404	745-747	2149-2151	787-789	1795-1797	802-804
Position 365	2462-2464	805-807	2209-2211	847-849	1855-1857	862-864
Position 368, 369	2471-2476	814-819	2218-2223	856-861	1864-1869	871-876
Position 455	2687-2689	1030-1032	2437-2439	1075-1077	2083-2085	1090-1092
Position 535	2864-2866	1207-1209	2623-2625	1261-1263	2272-2274	1279-1281
Position 537	2870-2872	1213-1215	2629-2631	1267-1269	2278-2280	1285-1287
Position 558	2924-2926	1267-1269	2683-2685	1321-1323	2329-2331	1336-1339

A. nidulans		A. niger	M. grisea
EST SEQ ID No. 10	cDNA SEQ ID No. 11	EST/cDNA SEQ ID No. 13	EST/cDNA SEQ ID No. 15
-	-	-	159-203
-	-	-	501-517
-	-	1-59	-
-	-	60-77	-
-	-	111-158	-
-	-	162-224	-
296-246	20-70	249-299	-
-	-	-	213-215
-	-	-	243-245
-	-	-	378-380
-	-	-	384-386
-	-	-	393-395
-	-	132-134	-

234-236	80-82	309-311	-
228-230	86-88	315-317	-
174-176	140-142	369-371	-

Preferably, the isolated polynucleotide comprises a PLC gene.

Preferably, the isolated polynucleotide comprises substantially a nucleotide sequence independently selected from at least one of the following:-

- 5 (i) bases 1658-2959 of SEQ ID No.1 or a complement, or a variant thereof;
- (ii) bases 1313-2733 of SEQ ID No.4 or a complement, or a variant thereof;
- (iii) bases 948-2376 of SEQ ID No.7 or a complement, or a variant thereof;
- 10 (iv) bases 141-315 of SEQ ID No.10 or a complement, or a variant thereof;
- (v) bases 1-404 of SEQ ID No.13. or a complement, or a variant thereof; and
- 15 (vi) bases 21-517 of SEQ ID No.15, or a complement, or a variant thereof.

Preferably, the isolated polynucleotide encodes a gene product, which gene product comprises at least one amino acid sequence independently selected from SEQ ID No.3, SEQ ID No.6, SEQ ID No.9, SEQ ID No.12, SEQ ID No.14 and SEQ ID No.16, or variants thereof.

- 20 According to a fourth aspect of the invention, there is provided an isolated polypeptide comprising substantially an amino acid sequence independently selected from at least one of SEQ ID No.3, SEQ ID No.6, SEQ ID No.9, SEQ ID No.12, SEQ ID No.14, and SEQ ID No.16, or variants thereof.

Preferably, the isolated polypeptide comprises a PLC.

- 25 Preferably, the polypeptide is encoded by a polynucleotide which polynucleotide comprises substantially a sequence independently selected from at least one of the following:-

- (i) bases 1658-2959 of SEQ ID No.1 or a complement, or a variant thereof;
- (ii) bases 1313-2733 of SEQ ID No.4 or a complement, or a variant thereof;
- 30 (iii) bases 948-2376 of SEQ ID No.7 or a complement, or a variant thereof;

- (iv) bases 141-315 of SEQ ID No.10 or a complement, or a variant thereof;
- (v) bases 1-404 of SEQ ID No.13. or a complement or, a variant thereof; and
- (vi) bases 21-517 of SEQ ID No.15 or a complement or, a variant thereof.

By the term "native amino acid/polynucleotide/polypeptide", we mean an amino acid, polynucleotide or polypeptide produced naturally from biological sources either *in vivo* or *in vitro*.

By the term "synthetic amino acid/polynucleotide/polypeptide", we mean an amino acid, polynucleotide or polypeptide which has been produced artificially or *de novo* using a DNA or protein synthesis machine known in the art.

By the term "recombinant amino acid/polynucleotide /polypeptide", we mean an amino acid, polynucleotide or polypeptide which has been produced using recombinant DNA or protein technology or methodologies which are known to the skilled technician.

The term "variant", and the terms "substantially the amino acid/polynucleotide/polypeptide sequence" are used herein to refer to related sequences. As discussed below such related sequences are typically homologous to (share percentage identity with) a given sequence, for example over the entire length of the sequence or over a portion of a given length. The related sequence may also be a fragment of the sequence or of a homologous sequence. A variant polypeptide may be encoded by a variant polynucleotide. A polynucleotide fragment will typically comprise at least 10 bases, such as at least 20, 30, 50, 100, 200, 500 or 1000 bases. A polypeptide fragment will typically comprise at least 10 amino acids, such as at least 20, 30, 50, 80, 100, 150, 200, 300, 400 or 500 amino acids.

By the term "variant", and the terms "substantially the amino acid/polynucleotide/polypeptide sequence", we mean that the sequence has at least 30%, preferably 40%, more preferably 50%, and even more preferably, 60% sequence identity with the amino acid/polynucleotide/polypeptide sequences of any one of the sequences referred to.

Calculation of percentage identities between different amino acid/polynucleotide/polypeptide sequences may be carried out as follows. A multiple alignment is first generated by the ClustalX program (pairwise parameters: gap opening 10.0, gap extension 0.1, protein matrix Gonnet 250, DNA matrix IUB;

multiple parameters: gap opening 10.0, gap extension 0.2, delay divergent sequences 30%, DNA transition weight 0.5, negative matrix off, protein matrix gonnet series, DNA weight IUB; Protein gap parameters, residue-specific penalties on, hydrophilic penalties on, hydrophilic residues GPSNDQERK, gap separation distance 4, end gap separation off). The percentage identity is then calculated from the multiple alignment as $(N/T)*100$, where N is the number of positions at which the two sequences share an identical residue, and T is the total number of positions compared. Alternatively, percentage identity can be calculated as $(N/S)*100$ where S is the length of the shorter sequence being compared. The amino acid/polynucleotide/polypeptide sequences may be synthesised *de novo*, or may be native amino acid/polynucleotide/polypeptide sequence, or a derivative thereof.

An amino acid/polynucleotide/polypeptide sequence with a greater identity than 65% to any of the sequences referred to is also envisaged. An amino acid/polynucleotide/polypeptide sequence with a greater identity than 70% to any of the sequences referred to is also envisaged. An amino acid/polynucleotide/polypeptide sequence with a greater identity than 75% to any of the sequences referred to is also envisaged. An amino acid/polynucleotide/polypeptide sequence with a greater identity than 80% to any of the sequences referred to is also envisaged. Preferably, the amino acid/polynucleotide/polypeptide sequence has 85% identity with any of the sequences referred to, more preferably 90% identity, even more preferably 92% identity, even more preferably 95% identity, even more preferably 97% identity, even more preferably 98% identity and, most preferably, 99% identity with any of the referred to sequences.

A sequence which is "substantially the amino acid/polynucleotide/peptide sequence" may be the same as the relevant sequence.

The above mentioned percentage identities may be measured over the entire length of the original sequence or over a region of 15, 20, 50 or 100 amino acids/bases of the original sequence. In a preferred embodiment percentage identity is measured with reference to SEQ ID Nos: 3, 6, or 9. Preferably the variant polypeptide has at least 40% identity, such as at least 60% or at least 80% identity with SEQ ID NOS: 3, 6 or 9 or a portion of SEQ ID NOS: 3, 6 or 9. Polynucleotide sequences which

encode such variant polypeptides are also preferred.

Alternatively, a substantially similar nucleotide sequence will be encoded by a sequence which hybridizes to the sequences shown in SEQ ID Nos. 1, 2, 4, 5, 7, 8, 10, 11, 13 or 15 or their complements under stringent conditions. By stringent
5 conditions, we mean the nucleotide hybridises to filter-bound DNA or RNA in 6x sodium chloride/sodium citrate (SSC) at approximately 45°C followed by at least one wash in 0.2x SSC/0.1% SDS at approximately 5-65°C. Alternatively, a substantially similar polypeptide may differ by at least 1, but less than 5, 10, 20, 50 or 100 amino acids from the sequences shown in SEQ ID Nos. 3, 6, 9, 12, 14 or 16. Such
10 differences may each be additions, deletions or substitutions. Due to the degeneracy of the genetic code, it is clear that any nucleic acid sequence could be varied or changed without substantially affecting the sequence of the protein encoded thereby, to provide a functional variant thereof. Suitable nucleotide variants are those having a sequence altered by the substitution of different codons that encode the same amino
15 acid within the sequence, thus producing a silent change.

Other suitable variants are those having homologous nucleotide sequences but comprising all, or portions of, sequence which are altered by the substitution of different codons that encode an amino acid with a side chain of similar biophysical properties to the amino acid it substitutes, to produce a conservative change. For
20 example small non-polar, hydrophobic amino acids include glycine, alanine, leucine, isoleucine, valine, proline, and methionine. Large non-polar, hydrophobic amino acids include phenylalanine, tryptophan and tyrosine. The polar neutral amino acids include serine, threonine, cysteine, asparagine and glutamine. The positively charged (basic) amino acids include lysine, arginine and histidine. The negatively charged
25 (acidic) amino acids include aspartic acid and glutamic acid. Certain organisms, including *Candida* are known to use non-standard codons compared to those used in the majority of eukaryotes. Any comparisons of polynucleotides and polypeptides from such organisms with the sequences given here should take these differences into account.

30 The accurate alignment of protein or DNA sequences is a complex process which has been investigated in detail by a number of researchers. Of particular importance is the trade-off between optimal matching of sequences and the introduction of gaps

to obtain such a match. In the case of proteins, the means by which matches are scored is also of significance. The family of PAM matrices (e.g., Dayhoff, M. et al., 1978, Atlas of protein sequence and structure, Natl. Biomed. Res. Found.) and BLOSUM matrices quantitate the nature and likelihood of conservative substitutions and are used in multiple alignment algorithms, although other, equally applicable matrices will be known to those skilled in the art. The popular multiple alignment program ClustalW, and its windows version ClustalX (Thompson et al., 1994, Nucleic Acids Research, 22, 4673-4680; Thompson et al., 1997, Nucleic Acids Research, 24, 4876-4882) are efficient ways to generate multiple alignments of proteins and DNA.

Frequently, automatically generated alignments require manual alignment, exploiting the trained user's knowledge of the protein family being studied, e.g., biological knowledge of key conserved sites. One such alignment editor programs is Align (<http://www.gwdg.de/~dhepper/download/>; Hepperle, D., 2001: Multicolor Sequence Alignment Editor. Institute of Freshwater Ecology and Inland Fisheries, 16775 Stechlin, Germany), although others, such as JalView or Cinema are also suitable.

Calculation of percentage identities between proteins occurs during the generation of multiple alignments by Clustal. However, these values need to be recalculated if the alignment has been manually improved, or for the deliberate comparison of two sequences. Programs that calculate this value for pairs of protein sequences within an alignment include PROTDIST within the PHYLIP phylogeny package (Felsenstein; <http://evolution.gs.washington.edu/phylip.html>) using the "Similarity Table" option as the model for amino acid substitution (P). For DNA/RNA, an identical option exists within the DNADIST program of PHYLIP.

Other modifications in protein sequences are also envisaged and within the scope of the claimed invention, i.e. those which occur during or after translation, e.g. by acetylation, amidation, carboxylation, phosphorylation, proteolytic cleavage or linkage to a ligand.

In one embodiment the variant polypeptide is able to complement the activity of the original PLC polypeptide in a fungal cell, for example the presence of the variant polypeptide in a fungal cell whose native equivalent PLC gene has been

rendered non-functional, may rescue a nutrient deficiency, reconstitute a nutrient scavenging pathway, restore physiological properties, or allow the cell to remain alive.

According to a fifth aspect of the present invention, there is provided a
5 polynucleotide defined in the first or third aspect or variant thereof, or a polypeptide defined in the second or fourth aspect or variant thereof, each being optionally modified, for use as a medicament or in diagnosis.

The polynucleotide or polypeptide may be modified prior to use, preferably to produce a derivative or variant thereof. The polynucleotide or polypeptide may be
10 derivatised. The polypeptide may be modified by epitope tagging, addition of fusion partners or purification tags such as glutathione *S*-transferase or maltose binding protein, addition of green fluorescent protein, covalent attachment of molecules including biotin or fluorescent tags, incorporation of selenomethionine, inclusion or attachment of radioisotopes or fluorescent/non-fluorescent lanthanide chelates. The
15 polynucleotide may be modified by methylation or attachment of digoxigenin (DIG) or by addition of sequence encoding the above tags, proteins or epitopes.

The polynucleotide defined in the first or third aspect, or the polypeptide defined in the second or fourth aspect, may not be modified or derivatised.

Preferably, the medicament is adapted to retard or prevent a fungal infection.
20 The fungal infection may be in human, animal or plant. The polynucleotide or polypeptide may be used for the development of a drug. The polynucleotide or polypeptide may be used in, or for the generation of, a molecular model of said polynucleotide or said polypeptide.

The invention provides a method of screening which may be used to identify
25 modulators of PLC genes or PLC polypeptides. In one embodiment of the method a candidate substance is contacted with a polynucleotide or polypeptide of the invention and whether or not the candidate substance binds or modulates the polynucleotide, or polypeptide is determined.

The modulator may increase or decrease expression of the polynucleotide, or
30 may promote (agonise) or inhibit (antagonise) the activity of the polypeptide. A therapeutic modulator (against fungal infection) will generally causes cause decreased expression of a PLC polypeptide or will inhibit the activity of a PLC

polypeptide.

The method may be carried out *in vitro* (inside or outside a cell) or *in vivo*. In one embodiment the method is carried out on a cell, cell culture or cell extract. The cell may or may not be a cell in which the polynucleotide or polypeptide is naturally present. The cell may or may not be a fungal cell, or may or may not be a cell of any of the fungi mentioned herein. The protein or polynucleotide may be present in a non-cellular form in the method, thus the protein may be in the form of a recombinant protein purified from a cell.

Whether or not a candidate substance modulates the activity of the polypeptide may be determined by providing the candidate substance to the polypeptide under conditions that permit activity of the polypeptide, and determining whether the candidate substance is able to modulate the activity of the polypeptide.

The activity which is measured may be any of the activities of the polypeptide of the invention mentioned herein, such as phospholipase C activity. In one embodiment the screening method comprises carrying out a phospholipase C assay in the presence and absence of the candidate substance to determine whether the candidate substance inhibits the phospholipase activity of the protein of the invention, wherein the phospholipase assay is carried out by contacting said protein with a phospholipase C substrate such as pNitro phenylphosphorylcholine (pNPPC), under conditions in which in the absence of the candidate substance the protein catalyses cleavage of the substrate.

In a preferred embodiment the inhibition of the phospholipase C reaction is measured by detecting the amount of pNPPC cleaved. This can be done spectroscopically by measurement at 405 nm (see Example 7).

Suitable candidate substances which can be tested in the above methods include naturally occurring or synthesised phospholipids or phospholipid derivatives, combinatorial libraries, defined chemical identities, peptide and peptide mimetics, oligonucleotides, natural product libraries and display libraries (e.g. phage display libraries). The candidate substances may be chemical compounds. Batches of the candidate substances may be used in an initial screen of, for example, ten substances per reaction, and the substances from batches which show inhibition tested individually. Furthermore, antibody products (for example, monoclonal and

polyclonal antibodies, single chain antibodies, chimeric antibodies and CDR-grafted antibodies) may also be tested.

According to a sixth aspect of the present invention, there is provided use of a polynucleotide defined in the first or third aspect or variant thereof, or a polypeptide
5 defined in the second or fourth aspect or variant thereof, optionally after modification, for the preparation of a medicament for the treatment of a fungal infection.

The polynucleotide or polypeptide may be modified prior to use, preferably to produce a derivative or variant thereof. The polynucleotide or polypeptide may be
10 derivatised. The polynucleotide or polypeptide may not be modified or derivatised.

Preferably, the medicament is adapted to retard or prevent a fungal infection. The treatment may comprise retarding or preventing fungal infection. Preferably, the drug and/or medicament comprises an inhibitor, preferably a PLC inhibitor. Preferably, the drug or medicament is adapted to inhibit expression and/or activity of
15 the polynucleotide or a fragment thereof, and/or the function of the polypeptide or a fragment thereof.

Preferably, the fungal infection comprises an infection by a filamentous fungus, more preferably an Ascomycete, and even more preferably, an organism independently selected from a group of genera consisting of *Aspergillus*; *Blumeria*;
20 *Candida*; *Cryptococcus*; *Encephalitozoon*; *Fusarium*; *Leptosphaeria*; *Magnaporthe*; *Phytophthora*; *Plasmopara*; *Pneumocystis*; *Pyricularia*; *Pythium*; *Puccinia*; *Rhizoctonia*; *Trichophyton*; and *Ustilago*.

Preferably, the fungal infection comprises an infection by an organism independently selected from a group of genera consisting of *Aspergillus* and
25 *Magnaporthe*.

Preferably, the fungal infection comprises an infection by an organism independently selected from a group of species consisting of *Aspergillus flavus*; *Aspergillus fumigatus*; *Aspergillus nidulans*; *Aspergillus niger*; *Aspergillus parasiticus*; *Aspergillus terreus*; *Blumeria graminis*; *Candida albicans*; *Candida*
30 *cruzei*; *Candida glabrata*; *Candida parapsilosis*; *Candida tropicalis*; *Cryptococcus neoformans*; *Encephalitozoon cuniculi*; *Fusarium solani*; *Leptosphaeria nodorum*; *Magnaporthe grisea*; *Phytophthora capsici*; *Phytophthora infestans*; *Plasmopara*

viticola; *Pneumocystis jiroveci*; *Puccinia coronata*; *Puccinia graminis*; *Pyricularia oryzae*; *Pythium ultimum*; *Rhizoctonia solani*; *Trichophyton interdigitale*; *Trichophyton rubrum*; and *Ustilago maydis*.

Preferably, the fungal infection comprises an infection by an organism
5 independently selected from a group of species consisting of *A. fumigatus*; *A. nidulans*; *A. niger*; and *M. grisea*.

According to a seventh aspect of the present invention, there is provided a method of detecting the presence of a fungal infection in an individual, said method comprising:-

- 10 (i) obtaining a sample from an organism; and
- (ii) detecting in the said sample the presence of a polynucleotide as defined by the first or third aspect, or variant thereof, or a polypeptide as defined by the second or fourth aspect, or variant thereof.

The individual may be a person (human) or animal (such as a mammal or bird) or
15 a plant. The individual may be of any of the species of animal mentioned below in regard to the therapeutic aspects of the invention. The fungal infection may arise from infection with an organism independently selected from a group of genera consisting of *Aspergillus*; *Blumeria*; *Candida*; *Cryptococcus*; *Encephalitozoon*; *Fusarium*; *Leptosphaeria*; *Magnaporthe*; *Phytophthora*; *Plasmopara*;
20 *Pneumocystis*; *Pyricularia*; *Pythium*; *Puccinia*; *Rhizoctonia*; *Trichophyton*; and *Ustilago*.

The fungal infection may arise from infection with an organism independently selected from a group of species consisting of *Aspergillus flavus*; *Aspergillus fumigatus*; *Aspergillus nidulans*; *Aspergillus niger*; *Aspergillus parasiticus*;
25 *Aspergillus terreus*; *Blumeria graminis*; *Candida albicans*; *Candida cruzei*; *Candida glabrata*; *Candida parapsilosis*; *Candida tropicalis*; *Cryptococcus neoformans*; *Encephalitozoon cuniculi*; *Fusarium solani*; *Leptosphaeria nodorum*; *Magnaporthe grisea*; *Phytophthora capsici*; *Phytophthora infestans*; *Plasmopara viticola*; *Pneumocystis jiroveci*; *Puccinia coronata*; *Puccinia graminis*; *Pyricularia oryzae*;
30 *Pythium ultimum*; *Rhizoctonia solani*; *Trichophyton interdigitale*; *Trichophyton rubrum*; and *Ustilago maydis*.

Preferably, the sample comprises a biological sample which, preferably, comprises nucleic acid and/or polypeptide. In one embodiment of the method the nucleic acid or polypeptide is purified (at least partially) from the sample before the detection is performed.

5 Where the organism is *Aspergillus fumigatus*, *Aspergillus nidulans* or *Aspergillus niger*, the sample may comprise sputum, bronchoalveolar lavage, urine, respiratory specimens, endotracheal aspirates, sterile specimens obtained by an invasive procedure such as vitreous tap, tympanocentesis, brain biopsy or aspiration, nasal or sinus specimens, blood, tissue or autopsy.

10 Where the organism is *Magnaporthe grisea* the sample may comprise rice leaf or rice stem.

Preferably, said detecting of the presence in the said sample of a polynucleotide as defined by the first or third aspect comprises use of at least one oligonucleotide pair adapted to be used for amplification of DNA, preferably genomic, more preferably,
15 fungal genomic DNA. The amplification may be PCR amplification.

Preferably, the PCR amplification employs at least one primer pair comprising a polynucleotide selected from the group consisting of:

Aspergillus fumigatus: SEQ ID Nos 64 + 65 (216bp product); SEQ ID Nos 66 + 67 (264 bp product), SEQ ID Nos 68 + 69 (211 bp product);
20 *Aspergillus nidulans*: SEQ ID Nos. 70 + 71 (147 bp product);
Aspergillus niger: SEQ ID Nos. 72 + 73 (139 bp product); and
Magnaporthe grisea: SEQ ID Nos. 74 + 75 (120 bp product)

Preferably, said detecting comprises subjecting the amplified DNA to size analysis, preferably, electrophoresis and, preferably, comparing the results to a
25 positive control and, preferably, a negative control. Said detecting may also comprise sequencing of the amplified DNA to demonstrate the correct sequence.

Preferably, said detecting of the presence in the said sample of a polypeptide as defined by the second or fourth aspect comprises use of a monoclonal or polyclonal antibody directed to part or all of the polypeptide as defined by the second or fourth
30 aspect.

According to an eighth aspect of the present invention, there is provided a recombinant DNA molecule or vector comprising a polynucleotide as defined in the

first or third aspect, or fragment or variant thereof.

The fragment may be a functional fragment of the polynucleotide. The fragment of the polynucleotide may comprise a polynucleotide sequence encoding any region of DNA shown in Tables I or II, or any combination thereof. The polynucleotide may encode a motif, region or domain or residue selected from Table II.

The recombinant DNA molecule may comprise an expression cassette. Preferably, the recombinant DNA molecule comprises an expression vector. Preferably, the polynucleotide sequence is operatively linked to an expression control sequence. A suitable control sequence may comprise a promoter, an enhancer etc.

According to a ninth aspect of the present invention, there is provided a cell containing a recombinant DNA molecule defined by the eighth aspect.

The cell may be transformed or transfected with the recombinant DNA molecule by suitable means. Preferably, the cell produces a recombinant protein of the invention.

Preferably, the cell of the ninth aspect produces a recombinant polypeptide. The recombinant polypeptide may comprise the isolated polypeptide defined by the second or fourth aspect, or fragment thereof. Preferably, the fragment is a functional fragment.

The invention also provides an organism which is transgenic for the polynucleotide of the invention (whose cells may be the same as the cells of the invention mentioned herein). Such an organism is typically a fungus, such as any genera or species of fungus mentioned herein. The organism may be a microorganism, such as a bacterium, virus or yeast. The organism may be a plant, animal (including birds and mammals), such as any of the animals mentioned herein.

The organism may be produced by introduction of the polynucleotide of the invention into a cell of the organism, and in the case of a multicellular organism allowing the cell to grow into a whole organism.

According to a tenth aspect of the present invention, there is provided a cell in which a polynucleotide defined by the first or third aspect, or variant thereof, or a polypeptide defined by the second or fourth aspect, or variant thereof, is non-functional and/or inhibited.

The cell may be a mutant cell. The cell is typically a fungal cell, such as of any genera or species of fungus mentioned herein. A preferred means of generating the cell is to modify the polynucleotide according to the first or third aspect, such that the polynucleotide is non-functional. This modification may be to cause a mutation, which disrupts the expression or function of a gene product. Such mutations may be to the nucleic acid sequences that act as 5' or 3' regulatory sequences for the polynucleotide, or may be a mutation introduced into the coding sequence of the polynucleotide. Functional deletion of the polynucleotide may be, for example, by mutation of the polynucleotide in the form of nucleotide substitution, addition or, preferably, nucleotide deletion.

The polynucleotide may be made non-functional and/or inhibited by:

- (i) shifting the reading frame of the coding sequence of the polynucleotide;
- (ii) adding, substituting or deleting amino acids in the protein encoded by the polynucleotide; or
- (iii) partially or entirely deleting the DNA coding for the polynucleotide and/or the upstream and downstream regulatory sequences associated with the polynucleotide.
- (iv) inserting DNA into the coding or non-coding regions.

A preferred means of introducing a mutation into a polynucleotide is to utilize molecular biology techniques specifically to target the polynucleotide which is to be mutated. Mutations may be induced using a DNA molecule. A most preferred means of introducing a mutation is to use a DNA molecule that has been especially prepared such that homologous recombination occurs between the target polynucleotide and the DNA molecule. When this is the case, the DNA molecule, which may be double stranded, may contain base sequences similar or identical to the target polynucleotide to allow the DNA molecule to hybridize to (and subsequently recombine with) the target.

It is also possible to provide a cell in which the polynucleotide is non-functional and/or inhibited without introducing a mutation into the PLC gene or its regulatory regions. This may be done by using specific inhibitors. Examples of such inhibitors include agents that prevent transcription of the polynucleotide, or prevent translation, expression or disrupt post-translational modification. Alternatively, the inhibitor may

be an agent that increases degradation of the gene product (e.g. a specific proteolytic enzyme). Equally, the inhibitor may be an agent which prevents the polynucleotide product from functioning, such as neutralizing antibodies (for instance an anti-PLC antibody). The inhibitor may also be an antisense oligonucleotide, an oligonucleotide
5 that mediates RNAi, or any synthetic chemical capable of inhibiting expression of the gene or the stability and/or function of the polypeptide. The inhibitor may also be a polypeptide which interacts with the PLC to prevent its function. The inhibitor may be an RNA molecule which causes inhibition by RNA interference. In one embodiment the antisense polynucleotide or RNA molecule which causes RNA interference are
10 examples of polynucleotides of the invention.

According to an eleventh aspect, there is provided an antibody exhibiting immunospecificity for a polypeptide of the second or fourth aspect, or fragment or variant thereof. The antibody may be used as a diagnostic reagent.

The antibody may be monoclonal or polyclonal, and may be raised in mouse,
15 rat, rabbit, chicken, turkey, horse, goat or donkey. The antibody may be raised against one or all of the PLC proteins together, or may be raised against proteolytic or recombinant fragments.

For the purposes of this invention, the term "antibody", unless specified to the contrary, includes fragments which bind a polypeptide of the invention. Such
20 fragments include Fv, F(ab') and F(ab')₂ fragments, as well as single chain antibodies. Furthermore, the antibodies and fragment thereof may be chimeric antibodies, CDR-grafted antibodies or humanised antibodies.

According to a twelfth aspect, there is provided a method for treating a fungal infection, the method comprising administering to an individual a polynucleotide
25 defined in the first or third aspect, or variant thereof, or a polypeptide defined in the second or fourth aspect, or variant thereof, each being optionally modified.

The polynucleotide may be modified prior to use, and may include derivatisation.

The polynucleotide or polypeptide may not be modified or derivatised prior to
30 administration to the individual. The method of treating may comprise antifungal therapy.

The individual may be a person (human), animal (such as a mammal or bird), or a plant. The animal is typically an agricultural animal such as a pig, cow, horse, sheep, goat, camel, chicken, duck, turkey or goose. The animal may be a cat or a dog.

5 The polypeptides, polynucleotides, vectors, cells or antibodies of the invention may be present in a substantially isolated form. They may be mixed with carriers or diluents which will not interfere with their intended use and still be regarded as substantially isolated. They may also be in a substantially purified form, in which case they will generally comprise at least 90%, e.g. at least 95%, 98% or 99%, of the
10 proteins, polynucleotides, cells or dry mass of the preparation.

According to a thirteenth aspect, there is provided a fungal PLC inhibitor.

Preferably, the inhibitor is adapted to inhibit, or render non-functional, a fungal PLC gene, or product thereof.

The fungal PLC gene may comprise a polynucleotide defined in the first or
15 third aspect, or variant thereof. The PLC gene product may comprise a polypeptide defined in the second or fourth aspect, or variant thereof.

Administration: The formulation of any of the therapeutic substances (e.g. proteins, polynucleotides or modulators) mentioned herein will depend upon factors such as the nature of the substance and the condition to be treated. Any such
20 substance may be administered in a variety of dosage forms. It may be administered orally (e.g. as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules), parenterally, subcutaneously, intravenously, intramuscularly, intrasternally, transdermally or by infusion techniques. The substance may also be administered as suppositories. A physician will be able to determine the required
25 route of administration for each particular patient.

Typically the substance is formulated for use with a pharmaceutically acceptable carrier or diluent. The pharmaceutical carrier or diluent may be, for example, an isotonic solution. For example, solid oral forms may contain, together with the active compound, diluents, e.g. lactose, dextrose, saccharose, cellulose, corn
30 starch or potato starch; lubricants, e.g. silica, talc, stearic acid, magnesium or calcium stearate, and/or polyethylene glycols; binding agents; e.g. starches, arabic gums, gelatin, methylcellulose, carboxymethylcellulose or polyvinyl pyrrolidone;

disaggregating agents, e.g. starch, alginic acid, alginates or sodium starch glycolate; effervescing mixtures; dyestuffs; sweeteners; wetting agents, such as lecithin, polysorbates, laurylsulphates; and, in general, non-toxic and pharmacologically inactive substances used in pharmaceutical formulations. Such pharmaceutical preparations may be manufactured in known manner, for example, by means of mixing, granulating, tableting, sugar-coating, or film coating processes.

Liquid dispersions for oral administration may be syrups, emulsions and suspensions. The syrups may contain as carriers, for example, saccharose or saccharose with glycerine and/or mannitol and/or sorbitol. Suspensions and emulsions may contain as carrier, for example a natural gum, agar, sodium alginate, pectin, methylcellulose, carboxymethylcellulose, or polyvinyl alcohol. The suspensions or solutions for intramuscular injections may contain, together with the active compound, a pharmaceutically acceptable carrier, e.g. sterile water, olive oil, ethyl oleate, glycols, e.g. propylene glycol, and if desired, a suitable amount of lidocaine hydrochloride.

Solutions for intravenous or infusions may contain as carrier, for example, sterile water or preferably they may be in the form of sterile, aqueous, isotonic saline solutions.

A therapeutically effective non-toxic amount of substance is administered. The dose may be determined according to various parameters, especially according to the substance used; the age, weight and condition of the patient to be treated; the route of administration; and the required regimen. Again, a physician will be able to determine the required route of administration and dosage for any particular patient. A typical daily dose is from about 0.1 to 50 mg per kg, preferably from about 0.1mg/kg to 10mg/kg of body weight, according to the activity of the specific inhibitor, the age, weight and conditions of the subject to be treated, the type and severity of the disease and the frequency and route of administration. Preferably, daily dosage levels are from 5 mg to 2 g.

Agricultural use: Modulators identified by the method of the invention may be administered to plants in order to prevent or treat fungal infections. The modulators are normally applied in the form of compositions together with one or more agriculturally acceptable carriers or diluents and can be applied to the crop area or

plant to be treated, simultaneously or in succession with further compounds.

The modulators of the invention can be applied together with carriers, surfactants or application-promoting adjuvants customarily employed in the art of formulation. Suitable carriers and diluents correspond to substances ordinarily employed in formulation technology, e.g. natural or regenerated mineral substances,
5 solvents, dispersants, wetting agents, tackifiers, binders or fertilizers.

A preferred method of applying the modulators of the present invention or an agrochemical composition which contains them is leaf application. The number of applications and the rate of application depend on the intensity of infection by the
10 fungus. However, the active ingredients can also penetrate the plant through the roots via the soil (systemic action) by impregnating the locus of the plant with a liquid composition, or by applying the compounds in solid form to the soil, e.g. in granular form (soil application). The active ingredients may also be applied to seeds (coating) by impregnating the seeds either with a liquid formulation containing active
15 ingredients, or coating them with a solid formulation. In special cases, further types of application are also possible, for example, selective treatment of the plant stems or buds.

The active ingredients are used in unmodified form or, preferably, together with the adjuvants conventionally employed in the art of formulation, and are
20 therefore formulated in known manner to emulsifiable concentrates, coatable pastes, directly sprayable or dilutable solutions, dilute emulsions, wettable powders, soluble powders, dusts, granulates, and also encapsulations, for example, in polymer substances. Like the nature of the compositions, the methods of application, such as spraying, atomizing, dusting, scattering or pouring, are chosen in accordance with the
25 intended objectives and the prevailing circumstances. Advantageous rates of application are normally from 50g to 5kg of active ingredient (a.i.) per hectare ("ha", approximately 2.471 acres), preferably from 100g to 2kg a.i./ha, most preferably from 200g to 500g a.i./ha.

The formulations, compositions or preparations containing the active
30 ingredients and, where appropriate, a solid or liquid adjuvant, are prepared in known manner, for example by homogeneously mixing and/or grinding active ingredients with extenders, for example solvents, solid carriers and, where appropriate, surface-

active compounds (surfactants).

Suitable solvents include aromatic hydrocarbons, preferably the fractions having 8 to 12 carbon atoms, for example, xylene mixtures or substituted naphthalenes, phthalates such as dibutyl phthalate or dioctyl phthalate, aliphatic hydrocarbons such as cyclohexane or paraffins, alcohols and glycols and their ethers and esters, such as ethanol, ethylene glycol, monomethyl or monoethyl ether, ketones such as cyclohexanone, strongly polar solvents such as N-methyl-2-pyrrolidone, dimethyl sulfoxide or dimethyl formamide, as well as epoxidized vegetable oils such as epoxidized coconut oil or soybean oil; or water.

The solid carriers used e.g. for dusts and dispersible powders, are normally natural mineral fillers such as calcite, talcum, kaolin, montmorillonite or attapulgite. In order to improve the physical properties it is also possible to add highly dispersed silicic acid or highly dispersed absorbent polymers. Suitable granulated adsorptive carriers are porous types, for example pumice, broken brick, sepiolite or bentonite; and suitable nonsorbent carriers are materials such as calcite or sand. In addition, a great number of pregranulated materials of inorganic or organic nature can be used, e.g. especially dolomite or pulverized plant residues.

Depending on the nature of the active ingredient to be used in the formulation, suitable surface-active compounds are nonionic, cationic and/or anionic surfactants having good emulsifying, dispersing and wetting properties. The term "surfactants" will also be understood as comprising mixtures of surfactants.

Suitable anionic surfactants can be both water-soluble soaps and water-soluble synthetic surface-active compounds. Suitable soaps are the alkali metal salts, alkaline earth metal salts or unsubstituted or substituted ammonium salts of higher fatty acids (chains of 10 to 22 carbon atoms), for example the sodium or potassium salts of oleic or stearic acid, or of natural fatty acid mixtures which can be obtained for example from coconut oil or tallow oil. The fatty acid methyltaurin salts may also be used.

More frequently, however, so-called synthetic surfactants are used, especially fatty sulfonates, fatty sulfates, sulfonated benzimidazole derivatives or alkylarylsulfonates. The fatty sulfonates or sulfates are usually in the form of alkali metal salts, alkaline earth metal salts or unsubstituted or substituted ammoniums salts and have a 8 to 22 carbon alkyl radical which also includes the alkyl moiety of alkyl

radicals, for example, the sodium or calcium salt of lignonsulfonic acid, of dodecylsulfate or of a mixture of fatty alcohol sulfates obtained from natural fatty acids. These compounds also comprise the salts of sulfuric acid esters and sulfonic acids of fatty alcohol/ethylene oxide adducts. The sulfonated benzimidazole derivatives preferably contain 2 sulfonic acid groups and one fatty acid radical containing 8 to 22 carbon atoms. Examples of alkylarylsulfonates are the sodium, calcium or triethanolamine salts of dodecylbenzenesulfonic acid, dibutyl-naphthalenesulfonic acid, or of a naphthalenesulfonic acid/formaldehyde condensation product. Also suitable are corresponding phosphates, e.g. salts of the phosphoric acid ester of an adduct of p-nonylphenol with 4 to 14 moles of ethylene oxide.

Non-ionic surfactants are preferably polyglycol ether derivatives of aliphatic or cycloaliphatic alcohols, or saturated or unsaturated fatty acids and alkylphenols, said derivatives containing 3 to 30 glycol ether groups and 8 to 20 carbon atoms in the (aliphatic) hydrocarbon moiety and 6 to 18 carbon atoms in the alkyl moiety of the alkylphenols.

Further suitable non-ionic surfactants are the water-soluble adducts of polyethylene oxide with polypropylene glycol, ethylenediamine propylene glycol and alkylpolypropylene glycol containing 1 to 10 carbon atoms in the alkyl chain, which adducts contain 20 to 250 ethylene glycol ether groups and 10 to 100 propylene glycol ether groups. These compounds usually contain 1 to 5 ethylene glycol units per propylene glycol unit.

Representative examples of non-ionic surfactants are nonylphenolpolyethoxyethanols, castor oil polyglycol ethers, polypropylene/polyethylene oxide adducts, tributylphenoxypolyethoxyethanol, polyethylene glycol and octylphenoxyethoxyethanol. Fatty acid esters of polyoxyethylene sorbitan and polyoxyethylene sorbitan trioleate are also suitable non-ionic surfactants.

Cationic surfactants are preferably quaternary ammonium salts which have, as N-substituent, at least one C₈-C₂₂ alkyl radical and, as further substituents, lower unsubstituted or halogenated alkyl, benzyl or lower hydroxyalkyl radicals. The salts are preferably in the form of halides, methylsulfates or ethylsulfates, e.g.

stearyltrimethylammonium chloride or benzyldi(2-chloroethyl)ethylammonium bromide.

The surfactants customarily employed in the art of formulation are described, for example, in "McCutcheon's Detergents and Emulsifiers Annual", MC Publishing Corp. Ringwood, New Jersey, 1979, and Sisely and Wood, "Encyclopaedia of Surface Active Agents," Chemical Publishing Co., Inc. New York, 1980.

The agrochemical compositions usually contain from about 0.1 to about 99% preferably about 0.1 to about 95%, and most preferably from about 3 to about 90% of the active ingredient, from about 1 to about 99.9%, preferably from about 1 to 99%, and most preferably from about 5 to about 95% of a solid or liquid adjuvant, and from about 0 to about 25%, preferably about 0.1 to about 25%, and most preferably from about 0.1 to about 20% of a surfactant. Whereas commercial products are preferably formulated as concentrates, the end user will normally employ dilute formulations.

According to a fourteenth aspect of the invention the phospholipases of the present invention may be used for degumming an aqueous carbohydrate solution or slurry to improve its filterability, particularly, a starch hydrolysate, especially a wheat starch hydrolysate which is difficult to filter and yields cloudy filtrates. The treatment may be performed using methods well known in the art.

Phospholipases of the present invention may be used in a process to reduce the phospholipid content in edible oil by treating the oil with the polypeptide to hydrolyse a major portion of the phospholipid and separating an aqueous phase containing the hydrolysed phospholipid from the oil. Such a process is applicable to the purification of any edible oil that contains phospholipid, e. g. , vegetable oil such as soybean oil, rapeseed oil, and sunflower oil. Prior to phospholipase treatment, the oil is preferably pre-treated to remove slime (mucilage), e. g. , by wet refining. Typically, the oil will contain 50-250 ppm of phosphorus as phospholipid at the beginning of the treatment with the phospholipase, and the treatment may reduce the phosphorus value to below 5-10 ppm.

The phospholipase treatment is conducted by dispersing an aqueous solution of the phospholipase, preferably as droplets with an average diameter below 10 μ m. The amount of water is preferably 0.5-5% by weight in relation to the oil. An

emulsifier may optionally be added. Mechanical agitation may be applied to maintain the emulsion. The phospholipase treatment can be conducted at a pH in the range of about 3.5 to about 5 to maximize the enzyme's performance, or a pH in the range of about 1.5 to about 3 (e. g. , 2-3) may be used in order to suppress the alkaline hydrolysis of triglycerides (saponification). The pH may be adjusted by adding citric acid, a citrate buffer, or hydrochloric acid. A suitable temperature is generally 30-70 C (particularly 30-45 C, e. g., 35-40 C). The reaction time will typically be 1-12 hours (e. g. , 2-6 hours). A suitable enzyme dosage will usually be 0.1-10 mg per liter (e. g. , 0.5-5 mg per liter). The phospholipase treatment may be conducted batchwise, e. g. , in a tank with stirring, or it may be continuous, e. g. , a series of stirred tank reactors. The phospholipase treatment is followed by separation of an aqueous phase and an oil phase. The separation may be performed by conventional means, e. g. , centrifugation. The aqueous phase will contain phospholipase, and the enzyme may be re-used to improve the process economy. The treatment may be performed using any of the methods known in the art. See, for example, U. S. Patent No. 5,264, 367, EP-A-654,527, JP-A-2-153997.

According to a fifteenth aspect of the invention the proteins of the invention may be incorporated into a dough or baked product to improve one or more properties of the dough or the baked product obtained from the dough relative to a dough or a baked product in which the polypeptide is not incorporated. The phospholipase according to the invention may be added in any step of the dough preparation and may be added in one, two or more steps. The phospholipase according to the invention may be added to the ingredients of a dough that is kneaded and baked to make the baked product using methods well known in the art. See, for example, U. S. Patent No. 4,567, 046, EP-A-426, 211, JP-A-60-78529, JP-A-62-111629, and JP-A-63-258528.

The improved property may include, but is not limited to, increased strength of the dough, increased elasticity of the dough, increased stability of the dough, reduced stickiness of the dough, improved extensibility of the dough, improved flavour of the baked product, improved anti-staling of the baked product.

The improved property may be determined by comparison of a dough and/or a baked product prepared with and without addition of a polypeptide of the present invention.

Phospholipases of the present invention may be in any form suitable for the use in question, e. g. , in the form of a dry powder, agglomerated powder, or granulate, in particular a non-dusting granulate, liquid, in particular a stabilized liquid, or protected enzyme. Granulates and agglomerated powders may be prepared by conventional methods, e. g. , by spraying the phospholipase according to the invention onto a carrier in a fluid-bed granulator. The carrier may consist of particulate cores having a suitable particle size. The carrier may be soluble or insoluble, e. g. , a salt (such as NaCl or sodium sulphate), sugar (such as sucrose or lactose), sugar alcohol (such as sorbitol), starch, rice, corn grits, or soy. The phospholipase according to the invention and/or additional enzymes may be contained in slow-release formulations. Methods for preparing slow-release formulations are well known in the art. Adding nutritionally acceptable stabilizers such as sugar, sugar alcohol, or another polyol, and/or lactic acid or another organic acid according to established methods may for instance, stabilize liquid enzyme preparations.

The present invention also relates to methods for preparing a baked product comprising baking a dough obtained by a method of the present invention to produce a baked product. The baking of the dough to produce a baked product may be performed using methods well known in the art.

The present invention also relates to doughs and baked products, respectively, produced by the methods of the present invention.

All of the features described herein may be combined with any of the above aspects, in any combination. Embodiments of the invention will now be described by way of example, with reference to the accompanying drawings in which:-

Figure 1 illustrates a multiple sequence alignment of amino acid sequences corresponding to fungal, plant and bacterial PLCs, and acid phosphatases;

Figure 2 illustrates an electrophoretic gel on which has been run the products of PCR amplification of PLC-A, PLC-B and PLC-C genes from *A. fumigatus* genomic DNA. Lane 1, PLCA; lane 2, PLCB; lane 3, PLCC; lane 4, molecular weight

standards;

Figure 3 illustrates expression of PLC-A, PLC-B and PLC-C in wax moth larvae infected with *A. fumigatus*; M = marker; U = uninfected; E = early infection (48h infection); L = late infection (72h infection); H = infected with heat killed spores; 16 = *A. fumigatus* grown in SAB broth for 16h; - = no template control;

Figure 4 illustrates plasmid pZVK2;

Figure 5 illustrates an SDS-PAGE gel on which have been run fractions from the purification of soluble recombinant PLC-A from *E. coli*. E1-E3 shows elution of PLC-A (marked) from a Ni column with imidazole buffer. Molecular weight markers are also shown.

EXAMPLES

Example 1. Identification of PLC family genes in *Aspergillus fumigatus*

The TIGR *A. fumigatus* database (www.TIGR.org) was searched (tblastn) with the haemolytic phospholipase C precursor (PLC-H) from *Psuedomonas aeruginosa* (Accession PO 06200). Matches to three contigs were identified; contig 906 (1.2e-20), contig 804 (2.7e-10), and contig 51 (5.9e-07). The appropriate regions of the contig sequences were down-loaded from www.tigr.org and gene predictions carried out using Genscan (genes.mit.edu/GENSCAN.html; Settings; organism = vertebrate; Suboptimal exon cutoff = 1.00).

The *ab initio* prediction of genes from genomes is known to be an inaccurate process (Burset, M. and Guigó, 1996, Genomics, 34, 353-367) and this is particularly so when the programs used have not been specifically trained for the genome under examination (as is the case here). It is therefore necessary to carefully examine the predictions, to compare any predicted genes with any homologous proteins, and to exploit the operative's knowledge of fungal gene structure, and thus to arrive at an informed prediction. The predicted genes were therefore compared with known sequences of bacterial and plant PLCs, using blastp ([http:// blast.genome.ad.jp/](http://blast.genome.ad.jp/)), the multiple alignment program ClustalX (Thompson et al., 1997, Nucleic Acids Research, 24:4876-4882), and the alignment editor/ viewer Align (<http://www.gwdg.de/~dhepper/download/>; Hepperle, D., 2001: Multicolor Sequence

Alignment Editor. Institute of Freshwater Ecology and Inland Fisheries, 16775 Stechlin, Germany). This resulted in an optimal prediction for each gene.

Referring to Figure 1, there is shown a multiple alignment of the PLC-A, PLC-B and PLC-C proteins from *A. fumigatus* along with PLCs from *A. nidulans*, *A. niger* and *M. grisea*. PLCs from plants and bacteria and fungal acid phosphatases are also shown (see also Example 4).

Boxed regions refer to regions conserved between PLCs and acid phosphatases (boxes II, III, V, VI, X), regions conserved between PLCs only (boxes I, VII and IX) and regions conserved between fungal PLCs only (boxes IV and VIII). Highlighted amino acids outside the boxes indicate fungus-specific residues.

Fungal PLCs include: PLC-A, *A. fumigatus* PLC-A, SEQ ID No. 3; PLC-B, *A. fumigatus* PLC-B SEQ ID No. 6; PLC-C, *A. fumigatus* PLC-C, SEQ ID No. 9; Anid1, *A. nidulans* EST AA966809 SEQ ID No. 12; Anig1, *A. niger* EST BE758821, SEQ ID No. 13; Mpor1, *M. Grisea* EST BM862140, SEQ ID No. 15;

Plant PLCs include: *A. thaliana*, Athal1, T02648; Athal2, AB084295_1; Athal3, AB084293_1; Athal4, AB084294_1; Athal5, E86207; Athal6, AB084296_1; Rice, Rice1, AP003727_16;

Bacterial PLCs include: C_cresc, E87624, *Caulobacter crescens*; PHLA_MYCTU, *M. tuberculosis*; PHL-B_MYCTU, *M. tuberculosis*; PHLC_MYCTU, *M. tuberculosis*; PHL-D_MYCTU, *M. tuberculosis*; PHLN_BURPS, *B. pseudomallei*; PHL-N_PSEAE, *P. aeruginosa*; PLCH_PSEAE, *P. Aeruginosa*.

Acid phosphatases include: AP_Km, JC7179 *K. marxianus*; AP_Af, AF462065_1, *A. fumigatus*; AP_Pc, *P. chrysogenum*; AP_An, *A. niger*, PHOA_ASPNG.

Details of the sequence searches that identified the PLCs, and methods for the construction of multiple alignments are given in Example 4.1 hereinafter.

The alignment shown in Figure 1 indicates that the predicted genes were members of the PLC family. Thus, three *A. fumigatus* PLC genes were predicted, which have been named PLC-A, PLC-B and PLC-C, and the SEQ IDs for each gene are given in Table III.

Table III. Sequence ID Numbers for three *A. fumigatus* PLCs

	gDNA	cDNA	protein
PLC-A	SEQ ID No 1	SEQ ID No 2	SEQ ID No 3
PLC-B	SEQ ID No 4	SEQ ID No 5	SEQ ID No 6
PLC-C	SEQ ID No 7	SEQ ID No 8	SEQ ID No 9

As shown in Figure 1, PLC-A, PLC-B and PLC-C align well with each other and were of very similar length. In addition, all three PLCs have good signal peptides. The three proteins show ~50 % identity to each other, and ~20% identity to *P. aeruginosa* PLC, over the region which they align. PLC-A, PLC-B and PLC-C were shorter at the C-terminus than the *Pseudomonas* PLC. Further searching of the *A. fumigatus* database (www.tigr.org) with PLC-A, PLC-B or PLC-C did not show any other PLCs (tblastn; no match with E value less than 0.39).

The *Pseudomonas* PLC requires the presence of the PLC accessory protein, PlcR (accession code P40695), which may be involved in proteolytic activation of the protein. Searching the TIGR *A. fumigatus* database as above failed to demonstrate any homolog of PlcR. Furthermore, Blastp at <http://blast.genome.ad.jp/> (database = nr-aa; matrix, blosum62) failed to identify PlcR homologs in other bacteria or plants, despite the wide spread presence of PLC homologs in these organisms. Correct functioning of PLC-A, PLC-B and PLC-C in *A. fumigatus*, or in organisms other than *Pseudomonas*, is therefore unlikely to require a PlcR homolog.

A number of PLCs have been identified in bacteria other than *P. aeruginosa*, such as *Clostridium* α toxin (P15310), and *B. cereus* phospholipase (PS0197). Although these proteins show PLC activity, they do not show appreciable sequence homology to the *P. aeruginosa* and *A. fumigatus* PLC described here. Searches of the *A. fumigatus* genome with these sequences did not reveal any matches with E values < 0.86.

Example 2. Genomic Sequencing of PLC Genes

Following the above bioinformatic analyses, the genomic sequences of PLC-A, PLC-B and PLC-C were experimentally determined.

2.1 Bacterial and Fungal Strains

For bacterial cloning, *E. coli* strains Top10 (Invitrogen) and select96 (Promega) were used in accordance with manufacturers' instructions.

A. fumigatus clinical isolate AF293 (ref. No. NCPF7367; available to the public from the NCPF repository; Bristol, U.K.); the CBS repository (Belgium) or from Dr. David Denning's clinical isolate culture collection, Hope Hospital, Salford, U.K.) is the preferred strain according to the present invention. AF293 was isolated in 1993 from the lung biopsy of a patient with invasive aspergillosis and aplastic anaemia. It was donated by Shrewsbury PHLS.

2.2 Purification of *A. fumigatus* genomic DNA

To obtain mycelial material for genomic DNA isolation, approximately 10^7 *A. fumigatus* conidia were inoculated in 50 ml of Vogel's minimal medium and incubated with shaking at 200 rpm until late exponential phase (18-24 h) at 37°C. Mycelium was dried down onto Whatmann 54 paper using a Buckner funnel and a side-arm flask attached to a vacuum pump and washed with PBS/Tween. At this point, the mycelium could be freeze-dried for extraction at a later date.

The mycelium (fresh or freeze dried) was ground to a powder using liquid nitrogen in a -20°C cooled mortar. The ground biomass was transferred to 50 ml tubes on ice up to the 10 ml mark. An equal volume of extraction buffer (0.7 M NaCl; 0.1 M Na₂SO₃; 0.1 M Tris-HCl pH 7.5; 0.05 M EDTA; 1%(w/v) SDS; pre-warmed to 65°C) was then added to each tube, mixed thoroughly with a pipette tip and incubated at 65°C for 20 minutes in a water bath. A volume of chloroform/isoamyl alcohol (24:1) equivalent to the volume of the original biomass was then added to each tube, tubes were mixed thoroughly and incubated on ice for 30 min. Tubes were then centrifuged at 3,500 x g for 30 min and the aqueous phase carefully transferred to fresh 50 ml tubes without disturbing the interface.

An equal volume of chloroform/isoamyl alcohol (24:1) was added, the tubes vortexed and incubated on ice for 15 minutes. Tubes were then spun at 3,500 x g for 15 minutes. After this spin, if large amounts of precipitate were still present, the supernatant was removed and the chloroform:isoamyl alcohol step repeated. The supernatant was removed and placed in clean sterile Oak Ridge tubes. An equal

volume of isopropanol was added and mixed gently. Tubes were incubated at room temperature for at least 15 minutes. Tubes were then centrifuged at 3,030 x g for 10 minutes at 4°C to pellet the DNA. The supernatant was removed and the pellet allowed to air dry for 10-25 minutes. The pellet was suspended in 2 ml sterile water.

5 1 ml of 7.5 M ammonium acetate was added, mixed and incubated on ice for 1 hour. Tubes were centrifuged at 12,000 x g for 30 min, the supernatants transferred to a fresh tube and 0.54 volumes of isopropanol were added, mixed and incubated at room temperature for at least 15 minutes. Tubes were then centrifuged at 5,930 x g for 10 min, the supernatant was removed and the pellet washed in 1 ml of 70% ethanol.

10 Tubes were centrifuged at 5,930 x g for 10 min and all the ethanol was removed. The pellet was air dried for 20-30 minutes at room temperature and suspended in 0.5-1.0 ml of TE (10 mM Tris-HCl pH 7.5; 1mM EDTA) Finally, the DNA was treated with RNase A (5 µl of 1mg/ml stock).

15 2.3 Primer Design

Primers were designed to the upstream and downstream regions of the *A. fumigatus* AF293 PLC-A, PLC-B and PLC-C genes.

PLC-A cloning primer pair: SEQ ID Nos. 17 (Mbplca-genF1: 1) + 18 (Mbplca-genF1: 3490) – product 3490 bases. Numbers in brackets after primer code

20 correspond to 5' base of oligonucleotide SEQ ID No. 1.

PLC-B Cloning primer pair: SEQ ID Nos. 25 (mbplcb-genF1: 1) + 26 (mbplcb-genR1: 864 bases after end of gene) – product 3596 bases. Numbers in brackets after primer code correspond to 5' base of oligonucleotide SEQ ID No. 4.

PLC-C Cloning primer pair: SEQ ID Nos. 33 (mbplcc-genF1: 1483 bases upstream of start of gene) + 34 (mbplcc-genR1: 3062) – product ~3600 bases. Numbers

25 in brackets after primer code correspond to 5' base of oligonucleotide SEQ ID No. 7.

2.4 PCR Reactions

PCR reactions using the PLC-A, PLC-B or PLC-C primer pair were set up

30 using the Hifidelity 2x PCR master mix (ABGene, Blenheim Road, Epsom, Surrey, KT19 9AP, UK) according to the manufacturer's instructions. The following reagent concentrations and volumes were used:

Master mix 1

	Forward primer (10 pmol/ μ l)	1 μ l
	Reverse primer (10 pmol/ μ l)	1 μ l
5	AF293 genomic DNA (1.5-4 μ g/ml)	1 μ l
	Hifidelity Master Mix	12.5 μ l
	Molecular biology grade water,filter sterile (BDH)	9.5 μ l
	Total volume	25 μ l

- 10 The reactions were run using the following conditions on a Biometra personal PCR cyclor (Thistle Scientific Ltd, DFDS House, Goldie Road, Uddington, Glasgow, G71 6NZ):-

	Step1	95°C	5min
15	Step2	95°C	1min
	Step3	53°C	1min
	Step4	68°C	3min 30sec
	Step5	72°C	10min
	Step6	10°C	Hold

20

- 30 cycles of steps 2-4 were carried out and the PCR products were run on a gel. The product band (3.6 kb) was excised from the gel and purified using Qiagen's QIAquick Gel Extraction Kit (Qiagen Ltd, Boundary Court, Gatwick Road, Crawley, West Sussex, RH10 9AX, UK) according to the manufacturers instructions and
- 25 eluted into 40 μ l of sterile water (BDH molecular biology grade/filter sterile). The purified PCR products were examined on 1% agarose gels as shown in Figure 2.

Referring to Figure 2, lane 1, there is shown a band at ~3600 bp signifying the amplification of PLC-A (1302 bp) and a total of 2188 bp of upstream and downstream sequence.

- 30 Referring to Figure 2, lane 2, there is shown a band at ~3600 bp signifying the amplification of PLC-B (1420 bp) and a total of 2176 bp of upstream and downstream sequence.

Referring to Figure 2, lane 3, there is shown a band at ~3600 bp signifying the amplification of PLC-C (1428 bp) and a total of 2168 bp of upstream and downstream sequence.

5 2.5 Genomic DNA Cloning

The purified PCR products shown in Figure 2 (5.5 µl) were cloned into pGEM-Teasy (Promega) and then transformed into select 96 *E. coli* cells (Promega) according to the manufacturer's instructions. The transformation reactions were then plated onto LB agar plates containing ampicillin (100 µg/ml), 50 µl X-gal (4%) and
10 10 µl IPTG (100 mM). Following overnight incubation at 37°C, individual white colonies from each transformation were sub-cultured into LB broth containing ampicillin (100 µg/ml). After overnight incubation at 37°C with shaking, plasmids were extracted using Qiagen spin mini plasmid extraction kits according to the manufacturers instructions.

15

2.6 Genomic DNA Sequencing

Genomic DNA clones were sequenced by primer walking (MWG Biotech UK Ltd, Waterside House, Peartree Bridge, Milton Keynes, MK6 3BY). The experimentally determined sequences of PLC-A and PLC-C were identical in the
20 coding regions to those identified by bioinformatic analyses as shown as SEQ ID No.1 and SEQ ID No.7, respectively. In the case of PLC-B, SEQ ID No. 4, position 2208 was experimentally determined to be a T, although it is a C on the TIGR database. This mutation has no effect on the amino acid specified by the codon. Five single base changes in non-coding sequence were observed in PLC-B (G7->A, G52-
25 >A, A253->G, A980->G, C3030->T; in each case the experimentally determined sequence is given second).

Example 3. cDNA sequence and RACE for PLCs

The internal sequence of the PLC-A and PLC-B messages were determined by
30 cloning and sequencing cDNA. The 5' and 3' ends of the PLC-A gene and the 5' end of the PLC-B gene were determined by RACE (Rapid Amplification of cDNA Ends).

3.1 cDNA cloning and sequencing

3.1.1 *Preparation of A. fumigatus RNA and cDNA*: 20 ml of Vogels medium, 0.5 % (w/v) soybean phospholipid, and phosphate-free Vogels medium, 0.5 % (w/v) soybean phospholipid were inoculated with *A. fumigatus* spores and incubated with shaking at 37° C. Cultures were harvested after 24 hrs (phosphate-free Vogels medium) or 48 hrs (Vogels medium) by filtration, then washed twice with DEPC-treated water and transferred to a 50ml Falcon tube. Samples were frozen in liquid nitrogen and stored at -80°C until required.

To prepare RNA, fungal samples were ground to a fine powder under liquid nitrogen. RNA was then extracted using the Qiagen RNeasy Plant Mini Kit following the protocol for isolation of total RNA from filamentous fungi in the RNeasy Mini Handbook (06/2001, Pages 75-78, http://www.qiagen.com/literature/handbooks/rna/rnamini/1016272HBRNY_062001WW.pdf). The following modifications were used: At step 3, RLC was used as the lysis buffer of choice; At step 7, the Rneasy column was incubated for 5 min at room temperature after addition of RW1; The optional step 9a was carried out; At step 10, 30µl RNase-free water was added, the samples incubated for 10 min at room temperature, and then centrifuged; At step 11, the elution step was repeated to give a total volume of 60 µl RNA.

DNA contamination was removed from the RNA by the addition of Dnase, using 2µl DNase per µg RNA, in the presence of 10X DNase buffer and incubating at 37°C for 2h. DNase-treated RNA was cleaned up using the RNeasy Plant Mini Kit following the RNeasy Mini Protocol for RNA Cleanup (RNeasy Mini Handbook 06/2001, pages 79-81).

To synthesise cDNA from the above RNA the following reaction mixture was prepared: 100ng-1µg of DNA-free RNA, 3µl oligo (dT) (100ng/µl), and DEPC-treated water to a total volume of 42µl. Samples were incubated in a heat block at 65°C for 5 min after which they were allowed to cool slowly to room temperature. Then 2µl Ultrapure dNTPs, 1µl reverse transcriptase (Stratascript) and 5µl 10X reverse transcriptase reaction buffer (Stratascript) were added. Samples were incubated at 42°C for 1h, denatured at 90°C for 5 min and then cooled on ice.

3.1.2 Production of PLC cDNA constructs

PCR was carried out using the cDNA above to generate cDNA fragments corresponding to PLC-A and PLC-B. Primer pairs were designed to amplify the cDNA sequence for PLC-A and PLC-B (see below). PCR reactions were carried out
5 using the following reaction mixture and conditions:

Reaction mix:

- 12.5 µl 2x Hifidelity mastermix (ABgene)
- 1 µl primer A (10 pmol)
- 10 1 µl primer B (10 pmol)
- 5 µl template cDNA
- 5.5 µl nuclease-free water

The reactions are run using the following conditions:-

- | | | | |
|----|-------|------|------------|
| 15 | Step1 | 95°C | 5min |
| | Step2 | 95°C | 1min |
| | Step3 | 53°C | 1min 30sec |
| | Step4 | 68°C | 2min 30sec |
| | Step5 | 72°C | 10min |
| 20 | Step6 | 4°C | Hold |

40 cycles of steps 2-4 were carried out and the PCR products were purified using Qiagen's QIAquick PCR Purification Kit (Qiagen Ltd, Boundary Court, Gatwick Road, Crawley, West Sussex, RH10 9AX, UK) according to the manufacturers
25 instructions. The purified PCR products were examined on agarose gels.

cDNA fragments were then cloned in to the pGEM-Teasy vector (Promega). The plasmids were then transformed in to select 96 chemically competent *E.coli* cells and plated on to a pre-warmed LB, ampicillin, X-Gal selection plate. After an overnight incubation at 37°C, white ampicillin-resistant colonies were selected and
30 grown up in ampicillin containing LB medium. Plasmid DNA was isolated using the Plasmid Mini Kit (qiagen). Confirmation of the presence and correct orientation of the inserts was determined by restriction analysis. Constructs were sequenced from

the ends of the vector using standard sequencing primers SP6 and T7 at MWG Biotech UK Ltd, Waterside House, Peartree Bridge, Milton Keynes, MK6 3BY. Three clones were sequenced and the consensus sequence taken.

5 The appropriate primer pairs are given below, to correspond with A+B pairs in the method above.

PLC-A: SEQ ID 19 (Mbplca-rtF1: 1655) + 20 (Mbplca-rtR1: 2953). Numbers in brackets after primer code refer to position in SEQ ID No.1 at which oligonucleotides bind.

10 PLC-B: SEQ ID 27 (Mbplcb-rtF1: 1307) + 28 (Mbplcb-rtR1: 2718) Numbers in brackets after primer code refer to position in SEQ ID No. 4 at which oligonucleotides bind.

The cDNA sequence obtained for PLC-A and PLC-B agreed with the predicted cDNA sequence. cDNA sequence of PLC-A confirmed bases 18-1278 of SEQ ID No. 2. cDNA sequence of PLC-B confirmed bases 15-1336 of SEQ ID No. 5. The C->T substitution seen in the genomic DNA was not seen in the cDNA and may
15 therefore have been a sequencing error.

The cDNA sequence for PLC-C can be obtained in the same way as described above, using the primer pair SEQ ID 35 (Mbplcc-rtF1: 948) + 36 (Mbplcc-rtR1: 2372). The numbers in brackets after primer code refer to position in SEQ ID No. 7
20 at which oligonucleotides bind.

3.2 RACE

To determine the 5' and 3' ends of the PLC genes, RACE (Rapid Amplification of cDNA Ends) was carried out, using the GeneRacer™ Kit (Invitrogen; cat. No.
25 L1502-01), essentially as per manufacturers instructions.

3.2.1 Preparation of RNA: *A. fumigatus* biomass was prepared as described in 3.1.1. RNA was prepared using the FastRNA kit (QBIogene) following the manufacturer's instructions (Revision 6030-999-1J05) with the following amendments: At step 1 40
30 mg of biomass was used per extraction; At step 2, samples were processed for 20 seconds at speed 5, incubated on ice for 3 minutes, and processed again for 20 seconds at speed 5; At step 3 samples were centrifuged for 5 minutes; At step 5, 500

µl DIPS were added, mixed, and incubated at room temperature for 2 minutes. Samples were mixed again and incubated for a further 2 minutes; At step 6 two washes in 250 µl SEWS were carried out; At step 7, the pellet was dissolved in 50 µl SAFE buffer.

5

3.2.2 *RACE*: 1 µg total RNA prepared as described above was de-phosphorylated in a 10 µl reaction using 10 units of calf intestinal phosphate (CIP), 1 µl 10X CIP buffer and 40U RNaseOut™ (made up to 10 µl in DEPC water) at 50°C for 1 hour. Samples were then made up to 100 µl with DEPC water and the RNA extracted with 100 µl (25:24:1) phenol:chloroform: isoamyl alcohol. RNA was then precipitated by the addition of 2 µl mussel glycogen (10mg/ml), 10 µl 3M sodium acetate, pH5.2 and 220 µl 95% ethanol and the sample frozen on dry ice for 10 minutes. RNA was pelleted by centrifugation at 14,500 rpm for 20 minutes at 4°C, washed with 70% ethanol, air dried and re-suspended in 8 µl DEPC water.

15 De-phosphorylated RNA (7 µl) was de-capped in a 10 µl reaction with 0.5 U tobacco acid pyrophosphatase (TAP), 1 µl 10x TAP buffer and 40U RnaseOut™ for 1 hour at 37°C. RNA was extracted with phenol:chloroform and precipitated as above, and then re-suspended in 7 µl DEPC-treated water.

20 De-phosphorylated, de-capped RNA (7 µl) was added to the pre-aliquoted GeneRacer™ RNA Oligo (0.25 µg) and incubated at 65°C for 5 minutes. A 10 µl ligation reaction was then set up by the addition of 1 µl 10x ligase buffer, 1 µl 10mM ATP, 40U RnaseOut™ and 5U T4 RNA ligase and incubated at 37°C for 1 hour. RNA was extracted and precipitated as described previously and re-suspended in 11 µl DEPC-treated water.

25 First-strand cDNA was prepared by the addition of 1 µl GeneRacer™ Oligo dT primer and 1 µl dNTP mix (10mM each) to 10 µl ligated RNA and incubated at 65°C for 5 minutes. The following reagents were added to the 12 µl ligated RNA and primer mix; 4 µl 5x first strand buffer, 2 µl 0.1M DTT, 1 µl RNaseOut™ and 1 µl SuperScript™ II RT (200U/µl) and incubated first at 42°C for 50 minutes and then, to stop the reaction, at 70°C for 15 minutes. 2U RNase H was added to the reaction mix and incubated at 37°C for 20 minutes.

30

To amplify the 5' cDNA ends a 50 μ l PCR reaction was set up using 1 μ l of the RACE-ready cDNA prepared above, 3 μ l GeneRacer™ 5' primer (10 μ M), 1 μ l reverse gene-specific primer (10 μ M; see Table IV below), 1 μ l dNTP solution (10mM each), 2 μ l 50 mM MgSO₄, 5 μ l High Fidelity PCR buffer, 2.5 U Platinum®
 5 *Taq* DNA Polymerase High Fidelity and 36.5 μ l sterile water. Cycling parameters are given in Table IV below.

To amplify 3' ends a 50 μ l PCR reaction was set up using 1 μ l of the RACE-ready cDNA prepared above, 3 μ l GeneRacer™ 3' primer (10 μ M), 1 μ l forward gene-specific primer (10 μ M; see Table IV below), 1 μ l dNTP solution (10mM each),
 1.0 2 μ l 50 mM MgSO₄, 5 μ l High Fidelity PCR buffer, 2.5 U Platinum® *Taq* DNA Polymerase High Fidelity and 36.5 μ l sterile water. Cycling parameters are given in Table IV below:

1.5 Table IV. Cycling parameters for 5' and 3'RACE

PLC-A 5'RACE	PLC-B 5'RACE	PLC-A 3'RACE
94 °C 2min 1 cycle	94 °C 2min 1 cycle	94 °C 2min 1 cycle
94 °C 30s 5 cycles	94 °C 30s 5 cycles	94 °C 30s 5 cycles
72 °C 1min	72 °C 1min	72 °C 1min
94 °C 30s 5 cycles	94 °C 30s 5 cycles	94 °C 30s 5 cycles
72 °C 1min	72 °C 1min	72 °C 1min
94 °C 30s 25 cycles	94 °C 30s 25 cycles	94 °C 30s 25 cycles
65 °C 30s	62 °C 30s	66 °C 30s
68 °C 1min	68 °C 1min	68 °C 1min
68 °C 10 min	68 °C 10 min	68 °C 10 min
10 °C hold	10 °C hold	10 °C hold

Primers: SEQ ID Nos. 41 (1912), 42 (2269), 43 (2564)	Primers: SEQ ID Nos. 47 (1538), 48 (1690)	Primers: SEQ ID Nos. 44 (2244), 45 (2542), 46 (2614)
--	--	--

Numbers in brackets after primer SEQ IDs refer to position of 5' end of primer on SEQ ID No. 1 (PLC-A) or SEQ ID No. 4 (PLC-B).

5 The cDNA sequence for the 5' and 3' ends of the PLC-A message was identical to that given in SEQ ID No. 2. The 5' and 3' UTR were also determined; the longest message corresponding to bases 1427 to 3058 of SEQ ID No. 1. Taking together the cDNA sequencing and RACE data, the predicted cDNA sequence for PLC-A given in SEQ ID No. 2 was fully confirmed.

10 5' RACE confirmed the sequence for the 5' end of the PLC-B gene given as SEQ ID No. 5. 130 bases of 5' UTR were also identified, matching identically the corresponding sequence for SEQ ID No. 4.

The 3' end of the PLC-B gene and the 5' and 3' ends of the PLC-C gene are identified in the same way as described above, using the following primers:

15 PLC-B 3'RACE: SEQ ID Nos. 49 (2144), 50 (2538), and 51 (1738). Numbers in brackets refer to position of 5' base in SEQ ID No. 4.

PLC-C 5' RACE: SEQ ID Nos. 52 (1437), 53 (1884), 54 (1729) and 55 (1874). Numbers in brackets refer to position of 5' base in SEQ ID No. 7.

20 PLC-C 3'RACE: SEQ ID Nos. 56 (951), 57 (1411), 58 (1862) and 59 (2150). Numbers in brackets refer to position of 5' base in SEQ ID No. 7.

RACE confirmed the sequence for the 3' end of the PLC-B message and identified a 113 base 3' UTR. PCR conditions for PLC-B 3'RACE were as follows: 94°C 2 min; 94°C 30 sec, 72°C 1 min, 5 cycles; 94°C 30 sec, 70°C 1 min, 5 cycles; 94°C 30 sec, 62°C 30 sec, 68°C 1 min, 25 cycles; 68°C 10 min.

Example 4. Identification of other fungal PLCs

Homologs of the *A. fumigatus* PLCs may be identified in other organisms, particularly fungi, by means of bioinformatics analysis, degenerate PCR or Southern blotting.

5 Sequences identified by bioinformatics can be used to design primers which can in turn be used in PCR to generate DNA coding for the PLC homolog. Degenerate PCR enables sequence to be obtained for novel genes. This sequence information can be used to generate probes by PCR which can then used to screen cDNA or genomic libraries of the organism of interest to identify clones containing
10 the PLC homolog. Southern blots using fragments of PLC genes from one species as probes can identify the presence of a PLC homolog in the genome of a second species. The same probe can then be used to screen cDNA or genomic DNA libraries. Once clones corresponding to the novel genes have been identified they can be expressed for functional characterisation of the protein, and their expression during
15 infection studied.

4.1 Identification of PLC homologs by bioinformatics

A. fumigatus PLC-A sequence was used to search the non-redundant protein sequence database (nr) at <http://blast.genome.ad.jp> (blastp) to identify any related
20 genes. Typically these identified matches in plant and bacterial genomes (Table V). This indicates that the fungal PLCs have homologs in plants (*Arabidopsis* and *Oryza*), and bacteria (e.g. *Burkholderia pseudomallei*, *Caulobacter crescentus*, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*, *Ralstonia solanacearum*, *Streptomyces coelicolor*). There were no matches to human/mammalian proteins with
25 $E < 5.4$. A multiple alignment of fungal, plant and bacterial PLCs, as well as acid phosphatases, was generated (see Fig. 1). Multiple alignments are generated by means of programs such as ClustalX (Thompson et al., 1994, Nucleic Acids Research, 22, 4673-4680; Thompson et al., 1997, Nucleic Acids Research, 24, 4876-4882;) and/or using manual alignment editors such as Align
30 (<http://www.gwdg.de/~dhepper/download/>; Hepperle, D., 2001: Multicolor Sequence Alignment Editor. Institute of Freshwater Ecology and Inland Fisheries, 16775 Stechlin, Germany).

Table V. Results of BLAST search with PLC-A

Match No.	Code	Organism	E-value	Annotation
1	AP003727_16	<i>Oryza sativa</i>	8e-68	Hypothetical protein
2	T02648	<i>Arabidopsis thaliana</i>	5e-65	Probable phospholipase C
3	AB084296_1	<i>Arabidopsis thaliana</i>	2e-62	Phosphatidylglycerol specific phospholipase C
4	AY150477_1	<i>Arabidopsis thaliana</i>	6e-62	Unknown protein
..				
13	AY114143_1	<i>Burkholderia thailandensis</i>	2e-28	Non-hemolytic phospholipase C
..				
18	A26391	<i>Pseudomonas aeruginosa</i>	1e-25	phospholipase C

Searches of fungal gene and genome databases were specifically carried out to identify any fungal PLC homologs. Searching the EST database (www.ncbi.nlm.nih.gov/BLAST/; tblastn; database = est; organism = Fungi) identified one EST each from *A. nidulans* (AA966809; SEQ ID No. 10) *A. niger* (BE758821; SEQ ID No. 13), and *M. grisea* (BM862140; SEQ ID No. 15). When translated, the sequences from these ESTs aligned well with the bacterial, plant and fungal (*A. fumigatus*) PLCs (*A. nidulans* protein SEQ ID No. 12; *A. niger* protein SEQ ID No. 14; *M. grisea* protein SEQ ID No. 16; see Figure 1).

PLCs can also be identified using bioinformatics by searching the following databases, with the *A. fumigatus* PLC-A, PLC-B or PLC-C DNA or protein sequences, with the given parameters:

Neurospora crassa: www-genome.wi.mit.edu/annotation/fungi/neurospora;
blastp, E=1e-3, cutoff = 100; blosum62 matrix, or blastn with same E-value and
cutoff parameters;

Candida albicans: (alces.med.umn.edu/gbsearch/ybc.html);

5 *Cryptococcus neoformans*: www.genome.ou.edu/cneo_h99_blast.html;
www.genome.ou.edu/cneo_B3501_blast.html;

Saccharomyces cerevisiae: genome-www.stanford.edu/Saccharomyces/;
tblastn, blosum62, W=3);

Schizosaccharomyces pombe:
10 (www.sanger.ac.uk/Projects/S_pombe/blast_server.shtml).

Aspergillus nidulans: at [http://www-genome.wi.mit.edu/cgi-
bin/annotation/aspergillus/blast_page.cgi?organismName=Aspergillus](http://www-genome.wi.mit.edu/cgi-bin/annotation/aspergillus/blast_page.cgi?organismName=Aspergillus); tblastn vs
Aspergillus (monsanto); E = 1e-3; S2 cutoff = 100; Blosum62 matrix; filter = yes.

Other fungal genomes which may become available in the future may be
15 searched in a similar way.

Proteins identified from these approaches, or derived from genome sequences,
e.g., by gene prediction methods as described in Example 1, require further analysis
to ensure that they are PLCs, e.g. by alignment with the *A. fumigatus* PLCs described
here. In particular, it is important to distinguish between PLC and phospholipases,
20 which show some sequence similarity, but may be distinguished by the presence of
PLC-specific regions such as those described in Fig. 1, and/or by a greater similarity
to PLCs than acid phosphatases by blastn or blastp, or percentage identities of nucleic
acid or protein.

25 4.2 Identification of PLC homologs by degenerate PCR

4.2.1. *Preparation of genomic DNA from organism of interest*: Fungal cultures can be
prepared using methods suitable for particular species. For example, *Aspergillus* and
Candida species, *Cryptococcus neoformans*, *Fusarium solani* and *Trichophyton*
species are maintained on Sabouraud dextrose agar at 30-35°C; *Leptosphaeria*
30 *nodorum* on Malt agar medium (30 g/L malt extract; 15 g/L Bacto-agar, pH 5.5),
24.0°C; *Magnaporthe grisea* on Oatmeal agar (6.7 g/L agar, 53.3 g/L instant oatmeal)
25.0°C, or Cornmeal agar (Difco 0386), 26.0 C; *Phytophthora capsici* cultures are

maintained on on V-8 agar at 24°C; *Pyricularia oryzae* cultures are maintained on rice polish agar at 24°C under white fluorescent lights (12hr artificial day), and were subcultured every 7 - 14 days by the transfer of mycelial plugs to fresh plates; *Pythium ultimum* cultures are maintained on PDA at 24°C, and subcultured every 7
 5 days by the transfer of aerial mycelium to fresh plates with an inoculating needle; *Rhizoctonia solani* cultures are maintained on PDA at 24°C under fluorescent lights (12 h artificial day), and subcultured every 7 days by the transfer of mycelial plugs to fresh plates; *Ustilago maydis* cultures are maintained on PDY agar at 30°C in the dark, and subcultured by re-streaking.

10 Genomic DNA is prepared from cultures using standard methodologies, e.g. using the Qiagen DNeasy Plant Kit, or using methods described in Example 2.2.

4.2.2 PCR: Primers (SEQ ID Nos. 60 and 61) were designed on the PLC-specific regions given as boxes I and IX in Figure 1. However, those skilled in the art will
 15 appreciate that it may be necessary to try alternative primers. PCR reactions using the above primer pair are set up as follows:

12.5 µl 2x ReddyMix PCR mastermix (ABIGene)
 1 µl primer A (5 pmol)
 20 1 µl primer B (5 pmol)
 template gDNA (1.5-4 µg/ml)
 nuclease-free water to give a final volume of 25 µl

The reactions are run using the following conditions on a Biometra personal PCR
 25 cycler (Thistle Scientific Ltd, DFDS House, Goldie Road, Uddington, Glasgow, G71 6NZ):-

Step1	95°C	5min
Step2	95°C	1min
30 Step3	53°C	1min 30sec
Step4	68°C	2min 30sec
Step5	72°C	10min

Step6 4°C Hold

30 cycles of steps 2-4 are carried out. The PCR products are purified (to remove residual enzymes and nucleotides) using Qiagen's QIAquick PCR Purification Kit (Qiagen Ltd, Boundary Court, Gatwick Road, Crawley, West Sussex, RH10 9AX, UK) according to the manufacturers instructions and eluted into 40µl of sterile water (BDH molecular biology grade/filter sterile). The purified PCR products are examined on 1% agarose gels.

Those skilled in the art will appreciate that degenerate PCR may require variations in a number of parameters in the attempts to generate a product. These include primer concentration, template concentration, concentration of Mg²⁺ ions, elongation and annealing times, and annealing temperature. Variations in temperature can be accommodated by the use of a gradient PCR machine.

The purified PCR products are cloned into pPEM-Teasy (Promega) and then transformed into XL10-Gold® Kan ultracompetent *E. coli* cells according to the manufacturers instructions. The transformation reactions are then plated onto LB agar plates containing ampicillin (100 µg/ml), 50 µl X-gal (4%) and 10 µl IPTG (100 mM). Following overnight incubation at 37°C, individual white colonies from each transformation are sub-cultured into LB broth containing ampicillin (100 µg/ml). After overnight incubation at 37°C with shaking, plasmids are extracted using Qiagen spin mini plasmid extraction kits according to the manufacturers instructions and sent away for full-length sequencing.

4.3 Identification of PLC homologs by Southern Blotting

4.3.1 Digestion of genomic DNA and transfer to nylon membranes :Genomic DNA from the fungi of interest is prepared as described in 4.2.1 above, then digested with the appropriate restriction enzyme and run on 0.8 % agarose gel. The gel is then submerged in 250 mM HCl for no more than 10 mins, with shaking, at room temperature, after which the gel is rinsed with sterilised RO water.

Transfer of the DNA onto nylon membrane is carried out using 0.4 M NaOH. Transfer protocols and apparatus are well known and are described in e.g. Sambrook et al., (1989), Molecular Cloning, 2nd Edition., Cold Spring Harbor Laboratory Press.

After transfer, the DNA is fixed to the membrane by baking at 120°C for 30 min. The membrane can then be used immediately, or stored dry for future use.

4.3.2. *Preparation of probe*: Probes are generated either by restriction digests of PLC DNA or by PCR of an appropriate region. A suitable probe can be generated from PLC-A by PCR using the primer pair SEQ ID Nos. 60 and 61 and the methods give in 4.2.2.

1 µg DNA template is diluted in molecular biology water to a total volume of 16 µl, denatured in a boiling water bath for 10 mins, and quickly chilled on ice. 4 µl DIG-High Prime (1 mM dATP, 1mM dCTP, 1mM dGTP, 0.65 mM dTTP, 0.35 mM alkali-labile-digoxigenin-11-dUTP, 1 U/µl labelling grade Klenow enzyme, 5 x reaction buffer, in 50% (v/v) glycerol) is then added and the reaction incubated at 37°C for 20 hours, after which 2 µl of 200 mM EDTA pH 8.0 is added to terminate the labelling reaction. The labelling efficiency was estimated by comparison with DIG-labelled control DNA.

4.3.3. *Prehybridisation and Hybridisation*: The membrane is placed in a hybridisation tube containing 20 ml of prehybridisation solution (DIG Easy Hyb, Roche) per 100cm² of membrane surface area and prehybridised at 42°C for 2 hours in a hybridisation oven. The DIG- labelled probe is denatured by heating in a boiling water bath for 10 min and then chilled directly on ice. The probe is then diluted to ~200 ng/mL in hybridisation solution (Easy Hyb, Roche; at least 5 mL of hybridisation solution is required per hybridisation). The prehybridisation solution is discarded from the hybridization tube and the hybridisation solution containing the DIG-labelled probe added quickly. The hybridisation then proceeds overnight at a 42°C in the hybridisation oven. The optimum temperature is dependant on probe size and homology to target sequence and is determined empirically.

After hybridisation, the membrane is washed twice at 42°C, 5 mins per wash, with 50 mL of stringency wash solution (3 x SSC, 0.1% SDS; where 20 x SSC buffer is 3 M NaCl, 300mM sodium citrate, pH 7.0), followed by two washes at RT, 15 min per wash, in 50 mL stringency wash solution. The stringency of these washes

can be decreased by increasing the SSC concentration to 6 x SSC, 0.1% SDS and/or decreasing the wash temperatures.

4.3.4. *Detection*: The membrane is washed in 20 mL washing buffer (100mM Maleic acid, 150 mM NaCl; pH 7.5; 0.3% v/v Tween 20), and then incubated successively with the following; 20 mL blocking solution (1 % w/v blocking reagent for nucleic acid hybridisation, Roche, dissolved in 100mM maleic acid, 150 mM NaCl, pH 7), for 30 min at room temperature; Anti-DIG-alkaline phosphatase (Roche) diluted 1:5,000 in blocking buffer, 30 min at room temperature; Washing buffer, two washes. each of 15 min at room temperature; Detection buffer (100mM Tris-HCl, 100 mM NaCl; pH 9.5), 2 min at room temperature. The membrane is then removed, placed on top of an acetate sheet, and ~ 0.5 ml (per 100cm²) of CSPD or CDP-star added to the top of the membrane. A second sheet of acetate is then placed over the surface of the membrane, the assembly incubated for 5 min at room temperature and then sealed in a plastic bag. The assembly is then exposed to X-ray film for between 15 min and 1 hour. Optimal exposure time is determined empirically by increasing exposure time up to 24 hours.

The presence of a band on the gel is evidence of a PLC gene in the genomic DNA of interest. The molecular weight of the band depends on the size of the restriction fragment that contains the gene.

Example 5. PLC Expression during infection of wax moth larvae (*Galleria melonella*) infected with *A. fumigatus*

Wax moth larvae have been shown to be good model systems in which to study Candida infection (Cotter et al., 2000, FEMS Immunol Med Microbiol 27, 163-9; Brennan et al., 2002, FEMS Immunol Med Microbiol 34, 153-7). We have found that this insect system is also a good system in which to study Aspergillus infection (D. Law and J. Rooke, unpublished observations). We show that *A. fumigatus* PLC-A, PLC-B and PLC-C are induced during infection of wax moth larvae.

5.1 Growth and infection of wax-moth larvae

Spores of *A. fumigatus* (AF293), grown on Sabaraud Dextrose agar, were harvested and re-suspended in PBS/Tween 80. Spores were washed and the concentration adjusted to give an inoculum 100 x the dose sufficient to cause death in 90% of the test group 3-4 days after infection (for AF293 this is $5.0-7.0 \times 10^8$ cfu/ml). Inoculum concentration was estimated using an improved Neubauer haemocytometer counting chamber and confirmed by TVC enumeration.

Wax moth larvae were purchased from Livefood UK, Somerset, UK (www.livefood.co.uk), and were maintained in the dark at room temperature in wood shavings prior to infection. Healthy larvae (250mg +/- 50mg) were selected and incubated at 4°C for 10 minutes immediately prior to infection to immobilise them. Larvae were then injected through the cuticle of the left last pro-leg with 10 µl spore suspension (100x stock), using a sterile Hamilton syringe. Larvae were then transferred to a sterile Petri dish. The following controls were also established:

Larvae injected with 10ul PBS/Tween only; larvae injected with 10ul heat killed spores (killed by incubation for 20 min 100°C); larvae pierced but not injected; and untouched larvae. Larvae were incubated at 30°C and monitored at least twice daily. All treatments and controls were carried out on batches of 10 larvae. Larval deaths and general health condition was recorded every 24 hrs and dead or moribund larvae were removed from the test group.

5.2 Preparation of DNA-free RNA from *Aspergillus fumigatus*-infected wax moth larvae (*Galleria melonella*).

cDNA was prepared from the following sources: Uninfected larvae; larvae after 48h infection with *A. fumigatus* (early infection); larvae after 72h infection with *A. fumigatus* (late infection); larvae infected with heat-killed *A. fumigatus* spores; and *A. fumigatus* grown in SAB Sabaraud Dextrose agar) broth for 16hr.

Frozen larvae were ground to a fine powder under liquid nitrogen in a mortar and pestle previously baked at 22°C overnight treated with RNaseZAP, rinsed with DEPC-treated water (0.1% (v/v) DEPC, stirred for 1h and autoclaved for 1h) and cooled with liquid nitrogen. Ground sample was transferred to Eppendorf tubes (no more than 50 mg per tube) and total RNA extracted using the Qiagen RNeasy Plant

Mini Kit following the protocol for isolation of total RNA from filamentous fungi in the RNeasy Mini Handbook (06/2001, Pages 75-78, http://www.qiagen.com/literature/handbooks/rna/rnamini/1016272HBRNY_062001WW.pdf).

5 The following modifications were used: At step 3, 600 µl RLT was added to each 50 mg tissue and vortexed; At step 4, samples were centrifuged for 3 min at maximum speed; At step 6, all samples from the same tissues were applied to the same RNeasy column; At step 7, RNeasy column was incubated for 5 min at room temperature after addition of RW1; Optional step 9a was carried out twice; At step
10 10, 30 µl RNase-free water was added, samples incubated for 10 min at room temperature, and then centrifuged for 1 min at 14,000 RPM; At step 11, the elution step was repeated to give a total volume of 60 µl RNA. A sample of the RNA was run on a 1.5% agarose gel and the amount of RNA quantified using the molecular marker. RNA was then stored at -80°C.

15 A portion of the RNA was Dnase treated using 2 µl RNase-free DNase (Promega) per µg RNA, in the presence of 10X DNase buffer (Promega) at 37°C for 4h. The RNA was then cleaned up using the Qiagen RNeasy Plant Mini Kit following the RNeasy Mini Protocol for RNA Cleanup (RNeasy Mini Handbook 06/2001, pages 79-81), but including a further DNase treatment step during clean-up as in the
20 Rneasy handbook.

The following modifications were made: Optional step 5a was carried out; At step 6, 30µl RNase-free water was added, samples incubated for 10 min at room temperature and then centrifuged for 1 min at 14,000 RPM; At step 7, the eluate from step 6 was transferred onto the RNeasy column, incubated for 10 min at room
25 temperature, and then centrifuged for 1 min at 14,000 RPM. A sample of the DNase-treated RNA was run on an agarose gel, quantified and stored at -80°C.

5.3 Checking RNA samples for DNA contamination

To verify the absence of genomic DNA from the RNA samples, PCR was carried using primers that amplify the β-tubulin gene (SEQ ID Nos. 62 and 63). In the
30 absence of a reverse-transcription step, only gDNA will be detected and thus any gDNA contamination will be revealed. The following reaction mixture was set up:

- 12.5 μ l 2x ReddyMix PCR mastermix (ABIGene)
 1 μ l each primer (5 pmol)
 template gDNA (1.5-4 μ g /ml)
 5 nuclease-free water to give a final volume of 25 μ l

The reactions were run using the following conditions on a Biometra personal PCR
 cycler (Thistle Scientific Ltd, DFDS House, Goldie Road, Uddington, Glasgow, G71
 6NZ):-

10

Step1	95°C	5min
Step2	90°C	1min
Step3	51°C	1min
Step4	68°C	1min
15 Step5	68°C	10min
Step6	4°C	Hold

40 cycles steps 2-4

20

The presence of a PCR product indicated that genomic DNA is present and the
 sample was DNase-treated again. If the PCR was negative, no DNA was present in
 the sample.

5.4 Preparation of cDNA

25

300 μ g DNA-free RNA and 3 μ l oligo (dT) (100 ng/ μ l) were added to an RNase-free
 0.5 ml microcentrifuge tube, and made up a total volume of 42 μ l with DEPC-treated
 water. Samples were mixed and incubated in a heat block at 65°C for 5 min and then
 slowly cooled to room temperature. 2 μ l Ultrapure dNTPs (10 mM each, Clontech), 1
 μ l stratascript reverse transcriptase (Stratagene) and 5 μ l 10X reverse transcriptase
 reaction buffer were then added. The samples were incubated at 42°C for 1h,
 denatured at 90°C for 5 min and then cooled on ice. Samples were dispensed in 5-10
 μ l aliquots and stored at -20°C.

30

5.5 PCR to determine expression The following primer sets were used:

PLC-A: SEQ ID No. 23 PLC-AF2 (1732) + SEQ ID No. 24 PLC-AR2 (2216). Numbers in brackets refer to 5' base of oligonucleotide with reference to SEQ ID No. 1. The primer pair SEQ ID No. 21 (1822) and SEQ ID No. 22 (2334) may also be used.

PLC-B: SEQ ID No. 31 PLC-B-F2 (1525) and SEQ ID No. 32 PLC-B-R2 (2012). Numbers in brackets refer to 5' base of oligonucleotide with reference to SEQ ID No. 4. The primer pair SEQ ID No. 29 (1840) and SEQ ID No. 30 (2337) may also be used.

PLC-C: SEQ ID No. 39 PLC-C-F2 (1143) and SEQ ID No. 40 PLC-C-R2 (1643). Numbers in brackets refer to 5' base of oligonucleotide with reference to SEQ ID No. 7. The primer pair SEQ ID No. 37 (1274) and SEQ ID No. 38 (1769) may also be used.

For each primer set (i.e. for each gene of interest), and each cDNA sample the following reactions were set up:

12.5 µl 2x ReddyMix PCR mastermix (ABgene),
3ul cDNA (6 ng/µl),
1ul of each of the primer pair (at 10 pm/µl), and
7.5ul water.

For each primer set, a no-template control was set up with water instead of cDNA. PCR reactions were carried out in Biometra PCR machine:

Step 1, 95°C – 5 min

Step 2, 95°C – 1 min

Step 3, 55°C – 1.5 min

Step 4, 72°C – 2 min

Step 5, 72°C – 10 min

Step 6, 8°C - hold

39 cycles of steps 2, 3 and 4

Reaction products were then run on agarose gels and visualised by staining with ethidium bromide as shown in Figure 3. Referring to Figure 3, there is shown

expression data of PLC-A, PLC-B and PLC-C in wax moth larvae which have been infected with *A. fumigatus*. M = marker; U = uninfected; E = early infection (48h infection); L = late infection (72h infection); H = infected with heat killed spores; 16 = *A. fumigatus* grown in SAB broth for 16h; - = no template control.

- 5 All three genes are expressed during infection, although with differing profiles, as PLC-A expression increases from early to late infection, while PLC-B and PLC-C are expressed at lower levels in late infection. In addition, PLC-A, PLC-B and PLC-C were all seen to be expressed by *A. fumigatus* growing in culture.

10 Example 6. PLC Expression in infected mice

6.1 Infection of mice with *A. fumigatus* and extraction of tissues.

- Mice were infected with *Aspergillus fumigatus* and organs harvested as follows. Thirteen male CD1 mice were injected with the immunosuppressant cyclophosphamide (0.025 g/ml; 200 mg/kg) IV via the tail vein. After 72 hours, 15 twelve mice were injected with 0.15 ml *Aspergillus fumigatus* AF293 conidia (7.5×10^5 /ml). 11 hours after infection, four mice were sacrificed with an overdose of inhaled halothane. The brain, lungs, liver and kidney were removed, frozen by immersion in liquid nitrogen, and stored at -70°C . This was repeated at 24 and 48 hours after infection. RNA was prepared from mouse tissues as described for wax 20 moth larvae above (5.2 and 5.3).

6.2 Preparation of cDNA from DNA-free RNA.

- cDNA was prepared from DNA-free RNA using the Promega Reverse Transcription kit, following the protocol as supplied with the product (Technical Bulletin No. 099, 25 <http://www.promega.com/tbs/tb099/tb099.pdf>). In a modification to the protocol, the cDNA synthesis reaction was incubated for 60 min at 42°C rather than for the suggested 15 min. Samples were stored in 5-10 μl aliquots at -20°C .

6.3 PCR to determine expression

- 30 The following primer sets are used:

PLC-A: SEQ ID. No. 23 PLC-AF2 (1732) + SEQ ID. No. 24 PLC-AR2 (2216).
Numbers in brackets refer to 5' base of oligonucleotide with reference to SEQ ID No. 1.

PLC-B: SEQ ID No. 31 PLC-B-F2 (1525) and SEQ ID No. 32 PLC-B-R2
5 (2012). Numbers in brackets refer to 5' base of oligonucleotide with reference to SEQ ID No. 4.

PLC-C: SEQ ID No. 39 PLC-C-F2 (1143) and SEQ ID No. 40 PLC-C-R2
(1643). Numbers in brackets refer to 5' base of oligonucleotide with reference to SEQ ID No. 7.

10 For each primer set (i.e. for each gene of interest), and each cDNA sample the following reactions are set up:

12.5 µl 2x ReddyMix PCR mastermix (ABgene),

1.5 µl cDNA (50 ng/µl),

1.5 µl of each of the primer pair (at 10 pm/µl)

15 Water to a final volume of 25 µl

For each primer set, a no-template control is set up with water instead of cDNA.

PCR reactions are carried out in Biometra PCR machine:

Step 1, 95°C – 5 min

20 Step 2, 95°C – 1 min

Step 3, 55°C – 1.5 min

Step 4, 72°C – 2 min

Step 5, 72°C – 10 min

Step 6, 8°C - hold

25 39 cycles of steps 2, 3 and 4 are carried out.

Reaction products are then run on agarose gels and visualised by staining with ethidium bromide.

Example 7. Phospholipase assay

30 Phospholipase C was assayed in the supernatants from fungal cultures using the colorimetric assay of Kurioka and Matsuda (1976, *Anal Biochem* 75, 281-9). This assay is based on the specific hydrolysis of the colorimetric substrate p-

nitrophenylphosphorylcholine (pNPPC, Sigma N5879) by phospholipase C. Phospholipase C cleaves pNPPC at the phosphate bond to release p-nitrophenol and phosphorylcholine. The rate of reaction can then be followed by monitoring the accumulation of the coloured p-nitrophenol with time. This assay has been extensively in the study of PLC activity (Kurioka and Matsuda, 1976; Bennett *et al.*, 1998, *Microbiology* 144, 55-72; Florin-Christensen and Florin-Christensen, 1999, *Biochem-Mol-Biol-Int* 47, 283-92; Aragon *et al.* 2000, *Infect-Immun* 68, 1855-63).

A. fumigatus strain Af293 cultures were grown on Vogel's salt with 15 w/v glucose; Vogel's salt with 0.5% w/v soy bean phospholipids; and phosphate-free Vogel's salt with 0.5% w/v soy bean phospholipids, and supernatants harvested. Glucose cultures were grown for 24 hours. Cultures containing phospholipid should be grown for up to 48 hours.

The PLC assay was performed by mixing 800 µl of substrate 10 mM pNPPC in 0.25 M Tris-HCl, 60% (w/v) sorbitol pH 7.2, with 200 µl of filter sterilised culture supernatant in a 2 ml cuvette. Reaction mixtures were incubated at 37°C, and the absorbance was read at 1 h intervals at 410 or 485 nm, and plotted against time for each test sample. Calibration curves were plotted by comparing the absorbance of a known amount of control PLC with the unknown sample. *Clostridium perfringens* or *Bacillus cereus* PLC were used as positive controls.

A microplate assay was also used and consisted of 200 µl of substrate/buffer with 50 µl of culture supernatant or positive control PLC. Serial dilutions were performed across a 96 well microplate. The results are shown in Table VI below.

Little PLC activity was seen in Vogels + glucose (as shown in Table VI. However, the addition of phospholipid and the removal of phosphate from the medium resulted in the detection of PLC activity. This is probably due to induction of PLC expression in the fungus. PLC activity has therefore been demonstrated in *A. fumigatus*, and, in the absence of any PLC sequences in the *A. fumigatus* genome resembling other characterised PLCs (see Example 1), it is most likely that this activity is due to one or more of the PLCs (A, B and C) described herein.

Medium		Activity ¹
Vogels + glucose	24 h incubation	<0.25U/ml

Vogels + phospholipids	24 h incubation	1.4 U/ml
Vogels + phospholipids	48 h incubation	1.0U/ml
Vogels (phosphate free)+ phospholipids	48 h incubation	2.3U/ml

Table VI. PLC assays of *A. fumigatus* culture supernatants.

¹ 1 unit (1U) of PLC activity liberates 1.0 μ mole of water-soluble organic phosphorus from egg yolk L- α phosphatidylcholine per min at pH7.3 at 37 °C.

5

The methods described herein can be used for screening recombinant PLCs for inhibitors. 20 μ l recombinant PLC, produced as described in Example 9, are added to 10 μ l test compound and 20 μ l substrate in 96 or 384 well plates. Positive and negative substrate and compound controls are included as well as a no-enzyme control. All samples are assayed in duplicate. The colorimetric substrate described above can be used in the assay, although other colorimetric substrates could also be envisaged. Plates are incubated at 37°C and absorbances read at 1 hr intervals. This procedure would identify compounds which inhibited the cleavage of substrate by the PLC.

10

15

Recombinant PLC-A, described in Example 9, was assayed as above with the following modifications: To each well of a 96-well plate was added; 150 μ l of Tris/sorbitol buffer (with 20 mM CaCl₂), 40 μ l of substrate pNPPC (20mM), and 2 μ g or 10 μ g of purified protein. OD measurements were taken at 405nm. Plates were incubated at 37°C and read at 405 nm. Results are shown in Table VII and indicate that recombinant PLC-A cleaved pNPPC and was therefore functional.

20

	OD 405nm	
Time (hours)	2 μ g PLC-A	10 μ g PLC-A
0	0	0
0.5	0	0.03
1	0.051	0.26
2	0.115	0.9
3	0.258	2.669

5	0.497	4.651
6	0.569	>max ¹
7	0.712	>max
24	1.952	>max

Table VII. Enzyme assay for recombinant PLC-A. ¹“>max” indicates an OD reading beyond the range of the spectrophotometer.

- 5 Screening for PLC-A inhibitors can be carried out using the above assay, or assays based upon it, by including inhibitor substances in the assay and determining the extent to which the cleavage of pNCCP is reduced. Other substrates, which may be more suitable for assaying, can be determined by substituting them for pNCCP and determining whether they are cleaved more than pNCCP, and/or are cleaved more
- 10 rapidly.

Example 8. Method for detecting fungal infection

- The PLC sequences described in the invention may be exploited to diagnose fungal infections. Samples from patients potentially carrying an infection with *A. fumigatus*, *A. nidulans*, or *A. niger* or of rice leaves or stem potentially infected with *M. grisea*,
- 15 or other organisms are processed to extract DNA using the DNAeasy Tissue kit or QIAamp DNA Blood Mini kit(Quiagen, Crawley, UK), although other DNA preparation methods are available and suitable. Once DNA has been prepared, PCR reactions are set up as follows:

- 20 Reaction mix:
- 12.5 µl 2x ReddyMix PCR mastermix (ABgene)
 - 1 µl primer A (5 pmol)
 - 1 µl primer B (5 pmol)
 - 5 µl template DNA
- 25 5.5 µl nuclease-free water

The following primers are used (given in pairs to correspond with A and B in the above method):

Aspergillus fumigatus: PLC-A: SEQ ID No. 64 (2074) + SEQ ID No. 65 (2290). Numbers in brackets refer to position of 5' in SEQ ID No. 1.

PLC-B: SEQ ID No. 66 (1592) + SEQ ID No. 67 (1855). Numbers in brackets refer to position of 5' in SEQ ID No. 4.

5 PLC-C: SEQ ID No. 68 (1935) + SEQ ID No. 69 (2145). Numbers in brackets refer to position of 5' in SEQ ID No. 7.

A. nidulans: SEQ ID No. 70 (160) + SEQ ID No. 71 (306). Numbers in brackets refer to position of 5' in SEQ ID No. 10.

10 *A. niger*: SEQ ID No. 72 (235) + SEQ ID No. 73 (373). Numbers in brackets refer to position of 5' in SEQ ID No. 13.

M. grisea: SEQ ID No. 74 (234) + SEQ ID No. 75 (353). Numbers in brackets refer to position of 5' in SEQ ID No. 15.

Appropriate controls include; (i) template DNA but no primers; primers but no template (negative controls); (ii) cDNA encoding fungal PLC or DNA from cultured
15 fungi instead of patient DNA (positive control).

PCR reactions are run as follows:

Step1	95°C	5 min
Step2	95°C	1 min
20 Step3	53°C	1 min 30sec
Step4	72°C	1 min 30sec
Step5	72°C	10 min
Step6	4°C	Hold

25 30 cycles of steps 2-4 are carried out and the PCR products examined on agarose gels. The production of a band of the correct molecular weight is diagnostic of the presence of the particular fungus. It may be additionally necessary to carry out diagnostic restriction digests of the PCR products. If necessary, PCR products can be subcloned into a vector, such as pGEM-Teasy (Promega), and sequenced to verify
30 that the PCR products are from the appropriate fungus.

Alternatively, the presence of an infection with *A. fumigatus*, *A. nidulans*, *A. niger* or *M. grisea*, or other organisms can be detected by means of antibodies raised

against the fungal PLCs. One suitable means is the use of a capture ELISA. Here, microtitre plates are coated with a monoclonal antibody raised against the fungal PLC. Then the plates are incubated with diluted patient samples, or appropriate protein extracts of samples (particularly if the samples are biopsies). Plates are then
5 incubated with a polyclonal antibody (again against the PLCs). Finally, binding of the second antibody is detected by means of an enzyme-coupled or fluorescently-labelled antibody directed against the polyclonal. In practise, two monoclonal or polyclonal antibodies or various combinations may be used.

10 Example 9. Expression of recombinant PLCs and/or fragments

Recombinant PLC proteins or PLC fragments are expressed to enable detailed study of PLC function and as the starting point for the development of a high-throughput screen for inhibitory compounds. Initially, RNA was prepared from *A. fumigatus* cultures and used as the template for the production of PLC cDNA.

15

9.1 Preparation of *A. fumigatus* cDNA

A. fumigatus cDNA was prepared as described in Example 3.1.1.

9.2 Production of PLC cDNA constructs

20 PCR is carried out using the cDNA above to generate polynucleotides encoding PLC-A, PLC-B and PLC-C. The appropriate primer pairs are given here, to correspond with A+B pairs in the method below. PLC-A, SEQ ID Nos. 76 and 77; PLC-B, SEQ ID Nos. 78 and 79; PLC-C, SEQ ID Nos. 80 and 81; Primers with SEQ ID Nos. 76, 78, and 80 include the sequence CACCATG at the 5' end to enable cloning into a
25 pET101/D-TOPO vector (Invitrogen). PCR reactions are carried out using the following reaction mixture and conditions:

Reaction mix:

12.5 µl 2x ReddyMix PCR mastermix (ABgene)

30 1 µl primer A (5-10 pmol)

1 µl primer B (5-10 pmol)

5 µl template RNA

5.5 µl nuclease-free water

The reactions were run using the following conditions:-

	Step1	95°C	5min
5	Step2	95°C	1min
	Step3	53°C	1min 30sec
	Step4	68°C	2min 30sec
	Step5	72°C	10min
	Step6	4°C	Hold

10

30 cycles of steps 2-4 are carried out and the PCR products are purified using Qiagen's QIAquick PCR Purification Kit (Qiagen Ltd, Boundary Court, Gatwick Road, Crawley, West Sussex, RH10 9AX, UK) according to the manufacturer's instructions. The purified PCR products are examined on agarose gels.

15 The cDNA fragments are then cloned in to the pET101/D-TOPO vector (Invitrogen). The plasmids are then transformed in to TOP10 chemically competent *E. coli* cells and plated on to a prewarmed ampicillin (+) selection plate. After an overnight incubation at 37°C, ampicillin resistant colonies are selected and grown up in ampicillin containing LB medium. Plasmid DNA is isolated using the Plasmid
20 Mini Kit (qiagen). Confirmation of the presence and correct orientation of the inserts is determined by restriction analysis and sequencing of the construct.

Purified plasmid DNA, which has been confirmed to be of the correct sequence and orientation, is transformed into chemically competent BL21 Star (DE3) One Shot *E. coli* cells and grown overnight at 37°C. Protein expression is then induced by the
25 addition of isopropylthio-β-galactosidase (IPTG) and detected by SDS PAGE and western blotting using an anti-His antibody. Purification of the recombinant protein is then performed by metal affinity chromatography.

Alternative expression systems can be used for expression in bacteria, such as the glutathione S-transferase or mannose-binding fusion-protein system.

30

9.3 Further production of PLC cDNA constructs

PCR was carried out using cDNA prepared as described above to generate polynucleotides encoding PLC-A essentially corresponding to SEQ ID No. 3. PCR reactions were carried out using the following reaction mixture and conditions. All Reagents were present in the KOD kit (Novagen).

- 5
- 5 µl 10x PCR Buffer
 - 10 µl dNTPs (2mM)
 - 4 µl MgSO₄ (25mM)
 - 2 µl primer A (5 pmol) (SL_PLXA30F7; SEQ ID No. 83)
 - 10 2 µl primer B (5 pmol) (SL_PLXA30R5; SEQ ID No. 84)
 - 2 µl template cDNA
 - 23 µl nuclease-free water
 - 2 µl KOD Polymerase

- 15 PCR reactions were run using the following conditions:-

- | | | |
|----------|-------|-------------|
| Step1 | 94°C | 5 min |
| Step2 | 94°C | 1 min |
| Step3 | 52 °C | 1 min 30sec |
| Step4 | 68°C | 1 min 30sec |
| 20 Step5 | 68°C | 10 min |
| Step6 | 8°C | Hold |

- 40 cycles of steps 2-4 were carried out and the PCR products were purified using Qiagen's QIAquick PCR Purification Kit (Qiagen Ltd, Boundary Court, Gatwick
- 25 Road, Crawley, West Sussex, RH10 9AX, UK) according to the manufacturers instructions. The purified PCR products were examined on agarose gels.

- cDNA fragments were then cloned in to the pET30 Xa/LIC vector (Novagen), transformed into Nova Blue chemically competent *E. coli* cells, and plated on to a prewarmed kanamycin (+) selection plate. After an overnight incubation at 37° C,
- 30 kanamycin-resistant colonies were selected and grown up in LB medium (30 µg/ml kanamycin). Plasmid DNA was isolated using the Plasmid Mini Kit (Qiagen).

Confirmation of the presence and correct orientation of the inserts was determined by restriction analysis with NcoI and sequencing of the construct.

Purified plasmid DNA, which had been confirmed to be of the correct sequence and orientation, was transformed into chemically competent BL21 Star (DE3) One Shot *E. coli* cells and grown overnight at 30° C. 5 ml of an over-night culture were used to inoculate 100 ml of LB, 30 µg/ml kanamycin, and the cultures incubated at 30° C, 220 rpm until the cell density reached an optical density of 0.6 (approximately 3 hours). Expression of the recombinant protein was then induced with IPTG (1mM) for 4 hours.

Bacteria were harvested by centrifugation at 4500 rpm for 10 minutes and the pellets lysed in lysis buffer (10 ml Bugbuster (Novagen), 10 µl Benzonase (Novagen), 0.4 µl lysozyme (Novagen) and 100 µl 1M imadazole for 20 minutes at room temperature. Cells were then spun down at 16000g for 20' at 4° C and the supernatant, containing soluble recombinant protein, removed to a clean tube.

Supernatant was added to prewashed Ni-Nta resin at a concentration of 5-10 mg protein per ml of resin and allowed to bind for 1 hour at 4° C. Protein-resin mix was then poured into a column, washed twice in 4 ml of wash buffer (2.5 ml 1M phosphate buffer pH8 , 6.25 ml 4M NaCl, 1 ml 1M Imidazole pH8, 0.5 ml 10% Tween 20; made up to 50 ml in n.H₂O) and then eluted successively in 1 ml (E1), 2 ml (E2), and 1 ml (E3) fractions with elution buffer (250 µl 1M Phosphate Buffer pH8, 625 µl 4M NaCl, 1.25 ml 1M Imidazole pH8, 50 µl 10% Tween 20, made up to 5 ml in n.H₂O). Figure 5 shows the elution fractions, containing PLC-A, run on an SDS-PAGE gel and stained with coomassie. Fractions containing purified recombinant protein were desalted using a PD10 column (Amersham Pharmacia) equilibrated in 20 mM Tris.HCl pH 8.0. Protein fractions may be analysed by Western blotting using an S-tag HRP conjugate (Novagen).

Recombinant fragments of other PLCs can be generated using primer pairs designed against corresponding regions of the genes.

Example 10. Production of an antibody

Antibodies against the fungal PLCs will be of considerable use as diagnostic reagents (see example 4 above). As an immunogen, recombinant domains are used (as described in example 9). Alternatively, synthetic polypeptides encoding regions
5 either unique to the individual PLCs, or likely to provide cross-reactivity within a set of PLCs, a set of species, or a range of genera are used. Peptides may need to be conjugated to carrier proteins before immunization.

Preimmune sera from animals to be immunised are screened against the immunogen to ensure that there is no endogenous cross reactivity. Animals (typically
10 sheep, rabbits or mice) are then immunised. For polyclonal antibody production, the resulting sera is affinity purified using the immunogen cross-linked to a chromatography matrix. Alternatively, purification of the antibody fraction from the serum, e.g. using protein G or protein A cross-linked to a matrix, may be sufficient. Monoclonal antibody production proceeds by methods familiar to those skilled in the
15 art.

The specificities of the resulting polyclonal and/or monoclonal antibodies are checked by ELISA and/or western blotting using the immunogen, related constructs or whole cell lysates and extracts as targets. Negative controls, such as other PLCs, different constructs or different species are also employed to test specificity and/or to
20 determine the range of species and/or genus cross-reactivity.

Example 11. Production of fungi with PLC genes functionally disabled.

A BAC (bacterial artificial chromosome) clone library containing the *A. fumigatus* genome, partially digested with *Bam*HI and inserted into the vector pBACe3.6 was
25 purchased from the Sanger Centre, Cambridge, UK. The BAC clone containing the gene to be inactivated is identified by bioinformatics (BLAST searching of Sanger BAC and related databases) and the glycerol stock of the clone grown up in 50 ml LB, 20 µg/ml chloramphenicol at 37°C overnight. The overnight culture is centrifuged at 4,500 rpm for 15 min. The bacterial pellet is resuspended in 4 ml of
30 Buffer P1 (Qiagen plasmid miniprep kit) and then 4 ml of buffer P2 (Qiagen plasmid miniprep kit, lysis buffer) is added and mixed gently by inverting 3-6 times. Proteins and genomic DNA are precipitated by adding 4 ml of buffer P3 (Qiagen plasmid

miniprep kit, neutralizing buffer) and incubated on ice for 10 minutes. Following the centrifugation of the mixture at 4500 rpm for 30 min, the supernatant is transferred into a 50 ml falcon tube, an equal volume of phenol/chlorophorm (1:1) mixture is added, and the mixture centrifuged for 15 min at 4500 rpm. The supernatant is then
5 transferred into an oakridge tube and 0.7 volumes isopropanol are added. After mixing, the tube is centrifuged at 10,000 rpm (Beckman centrifuge, rotor JA-17) for 30 min at 4°C. The resulting pellet is washed with 2 ml 70% ethanol at the same speed. The resulting BAC DNA is resuspended in 100 µl buffer EB.

The transposition reaction is carried out as follows. 7 µl purified BAC, 1 µl
10 transposon pZVK2 (as illustrated in Figure 4, the sequence of which is SEQ ID No. 82 which is an engineered plasmid), containing the mosaic ends of pMOD2 (Epicenter), a kanamycin resistance gene and a Zeocin resistance gene under the control of fungal promoter) and 1 µl EZ:TN transposase (Epicenter) are incubated at 37°C for two hrs after which 1 µl stop solution (1% SDS) is added and the mixture
15 heated to 70°C for 10 minutes. Electrocompetent GeneHogs *E. coli* cells (Invitrogen) are then transformed with the transposed BAC, the cells plated onto LB agar, 25 µg/ml kanamycin, 20 µg/ml chloramphenicol, and plates incubated overnight at 37°C.

At least 96 colonies are picked and grown up in 96-well plates in 2xLB (double concentrated LB), 20 µg/ml chloramphenicol, at 37°C overnight. BAC DNA is then
20 purified using the Millipore montage 96 BAC KIT using a MWG ROBOSEQ 4200 robot. BACs containing the transposon inserted into the gene of interest are identified by PCRs both spanning the gene of interest and extending from the transposon into the BAC. Insertion into the gene of interest is manifested as an increase in product size. Southern blots are also carried out to ensure that the transposon has only
25 inserted once into the BAC.

The BAC is then linearised using a restriction enzyme determined to cut in the vector backbone but not the BAC DNA, and used to transform *A. fumigatus* strain Af293. *A. fumigatus* (haploid) protoplasts are prepared using 5% Glucanex (Novo Nordisk A/S) solution (in 0.6 M KCl) and shaking for 2 h at 80 rpm in 30 °C. The
30 protoplasts are washed with 0.6 M KCl and then with STC (Sorbitol, Tris, CaCl₂). The washed protoplasts are diluted in STC to 10⁵ /ml and 100 µl transferred into 14 ml falcon tubes. 7 µl of linearised BAC are added to the tube and the whole mixture

incubated on ice for 20 min. Transformation is carried out by adding 200 μ l of PEG 8000 solution (60%w/v, pH 7.5) dropwise over 2 min and then adding 800 μ l PEG. The mixture is left at room temperature for 20 min. Transformed protoplasts are washed with STC, resuspended in 1 ml STC, spread onto CM-sorbitol- Zeocin (250 μ g/ml) plates and incubated at 37^o C.

After 4-10 days incubation, zeocin resistant colonies are picked and checked for presence of the knocked-out gene by PCR using primers which specifically amplify the whole gene of interest. Usually 10-20 transformants are checked. The ectopic integration of the BAC gives two bands by PCR, one for the endogenous gene and one for the BAC/transposon construct, which has a higher molecular weight. Replacement of the endogenous gene with the transposon-modified gene results in a single band of higher molecular weigh by PCR. If none of the transformants show the disrupted endogenous gene, the gene of interest may be essential, with the knock-out cells having died and only cells where replacement was unsuccessful surviving. In this case, the transformation is carried out on diploids using the same method of transformation. Essentiality of the gene is then tested by rehaploidisation, and examining the segregation pattern in haploids.

The reader's attention is directed to all papers and documents which are filed concurrently with or previous to this specification in connection with this application and which are open to public inspection with this specification, and the contents of all such papers and documents are incorporated herein by reference.

All of the features disclosed in this specification (including any accompanying claims, abstract and drawings), and/or all of the steps of any method or process so disclosed, may be combined in any combination, except combinations where at least some of such features and/or steps are mutually exclusive.

Each feature disclosed in this specification (including any accompanying claims, abstract and drawings), may be replaced by alternative features serving the same, equivalent or similar purpose, unless expressly stated otherwise. Thus, unless expressly stated otherwise, each feature disclosed is one example only of a generic series of equivalent or similar features.

The invention is not restricted to the details of the foregoing embodiment(s). The invention extends to any novel one, or any novel combination, of the features disclosed in this specification (including any accompanying claims, abstract and drawings), or to any novel one, or any novel combination, of the steps of any method
5 or process so disclosed.

CLAIMS

1. A PLC polynucleotide that comprises:
- 5 (i) a nucleotide sequence independently selected from at least one of SEQ ID NOS: 1, 2, 4, 5, 7, 8, 10, 11, 13 or 15, or complement thereof; or
- (ii) a nucleotide sequence which has sequence identity with (i); or
- (iii) a nucleotide sequence which is a fragment of (i) or (ii);
- wherein (ii) or (iii) optionally encode a polypeptide which has phospholipase activity.
- 10
2. A PLC polypeptide that comprises:
- (i) an amino acid sequence independently selected from at least one of SEQ ID NOS: 3, 6, 9, 12, 14 or 16; or
- (ii) an amino acid sequence which has sequence identity with (i); or
- 15 (iii) an amino acid sequence which is a fragment of (i) or (ii);
- wherein (ii) or (iii) optionally have phospholipase activity.
3. A PLC polypeptide, optionally having phospholipase activity, wherein said polypeptide comprises an amino acid sequence independently selected from at least
- 20 one of the following:-
- (i) Fungal-specific predicted active site residues shown at position 236 and/or 265 and/or 279 and/or 365 and/or 368 and/or 369 and/or 535 and/or 537 of rows PLC-A/PLC-B/PLC-C/Anid1/Anig1/Mpor1 as shown in Figure 1, or variant thereof;
- 25 (ii) Fungal PLC-specific residues shown at positions 98 and/or 145 and/or 147 and/or 150 and/or 320 and/or 321 and/or 326 and/or 327 and/or 345 and/or 455 and/or 558 of rows PLC-A/PLC-B/PLC-C/Anid1/Anig1/Mpor1 as shown in Figure 1, or variant thereof.
- 30 4. A PLC polypeptide, optionally having phospholipase activity, wherein the said polypeptide comprises a motif/domain independently selected from at least one of the following:-

- (i) a predicted active site region comprising an amino acid sequence substantially as set out in rows PLC-A, PLC-B, PLC-C, Anid1, Anig1 or Mpor1 in boxes II, III, V, VI or X as shown in Figure 1, or a variant thereof;
- 5 (ii) a PLC-specificity region comprising an amino acid sequence substantially as set out in rows PLC-A, PLC-B, PLC-C, Anid1, Anig1 or Mpor1 in boxes I, VII or IX as shown in Figure 1, or a variant thereof;
- 10 (iii) a fungal PLC-specific region comprising an amino acid sequence substantially as set out in rows PLC-A, PLC-B, PLC-C, Anid1, Anig1 or Mpor1 in boxes IV or VIII as shown in Figure 1, or a variant thereof;

5: A PLC polypeptide according to any of claims 2 to 4, wherein the polypeptide
 15 is encoded by a polynucleotide which comprises substantially a sequence independently selected from at least one of the regions and sequences given in the columns "gDNA/EST" in Table I, or a complement, or variant thereof.

6. A PLC polynucleotide according to claim 1, wherein the isolated
 20 polynucleotide encodes a fungal PLC comprising substantially the amino acid sequence as shown as SEQ ID NOS: 3, 6, 9, 12, 14 or 16, or variant thereof.

7. A PLC polypeptide according to any one of claim 2-4, wherein the polypeptide
 25 is encoded by a polynucleotide comprising substantially a sequence independently selected from at least one of the sequences given as SEQ ID NOS: 1, 2, 4, 5, 7, 8, 10, 11, 13 or 15, or a complement, or variant thereof.

8. A polynucleotide according to claims 1 or 6 comprising a PLC gene isolated from
 30 an organism independently selected from a group of species consisting of *Aspergillus flavus*; *Aspergillus fumigatus*; *Aspergillus nidulans*; *Aspergillus niger*; *Aspergillus parasiticus*; *Aspergillus terreus*; *Blumeria graminis*; *Candida albicans*; *Candida cruzei*; *Candida glabrata*; *Candida parapsilosis*; *Candida tropicalis*; *Cryptococcus*

neoformans; *Encephalitozoon cuniculi*; *Fusarium solani*; *Leptosphaeria nodorum*; *Magnaporthe grisea*; *Phytophthora capsici*; *Phytophthora infestans*; *Plasmopara viticola*; *Pneumocystis jiroveci*; *Puccinia coronata*; *Puccinia graminis*; *Pyricularia oryzae*; *Pythium ultimum*; *Rhizoctonia solani*; *Trichophyton interdigitale*;
5 *Trichophyton rubrum*; and *Ustilago maydis*.

9. A PLC polypeptide according to claims 2, 3, 4, 5, 7 or 9 isolated from an organism independently selected from a group of species consisting of *Aspergillus flavus*; *Aspergillus fumigatus*; *Aspergillus nidulans*; *Aspergillus niger*; *Aspergillus*
10 *parasiticus*; *Aspergillus terreus*; *Blumeria graminis*; *Candida albicans*; *Candida cruzei*; *Candida glabrata*; *Candida parapsilosis*; *Candida tropicalis*; *Cryptococcus neoformans*; *Encephalitozoon cuniculi*; *Fusarium solani*; *Leptosphaeria nodorum*; *Magnaporthe grisea*; *Phytophthora capsici*; *Phytophthora infestans*; *Plasmopara viticola*; *Pneumocystis jiroveci*; *Puccinia coronata*; *Puccinia graminis*; *Pyricularia*
15 *oryzae*; *Pythium ultimum*; *Rhizoctonia solani*; *Trichophyton interdigitale*; *Trichophyton rubrum*; and *Ustilago maydis*.

10. Use of a polynucleotide or polypeptide as defined in any one of the preceding claims, or variant thereof, each being optionally modified, for drug research
20 purposes.

11. Use of a polynucleotide or polypeptide as defined in any one of the preceding claims to identify or obtain a modulator of a PLC gene or PLC polypeptide.

25 12. A method of identifying a modulator of a PLC polypeptide comprising contacting a candidate substance with a polypeptide according to any one of claims 2-5, 7, 8 or 9 and determining whether the candidate substance binds or modulates the polypeptide.

30 13. A method according to claim 12 which further comprises formulating the identified modulator into a pharmaceutical composition.

14. A method according to claim 12 which further comprises using the identified modulator as an anti-fungal agent.
15. Use of a polynucleotide or polypeptide as defined in any one of claims 1 to 9,
5 or variant thereof, or a modulator thereof, for the preparation of a medicament for the treatment of fungal infection.
16. Use of a modulator according to claim 15, wherein the modulator is adapted to inhibit expression and/or activity of the PLC polynucleotide or a fragment thereof,
10 and/or the function of an PLC polypeptide or a fragment thereof.
17. Use according to claim 15 or claim 16, wherein the fungal infection comprises an infection by an organism independently selected from a group of genera consisting of *Aspergillus*; *Blumeria*; *Candida*; *Cryptococcus*; *Encephalitozoon*;
15 *Fusarium*; *Leptosphaeria*; *Magnaporthe*; *Phytophthora*; *Plasmopara*; *Pneumocystis*; *Pyricularia*; *Pythium*; *Puccinia*; *Rhizoctonia*; *Trichophyton*; and *Ustilago*.
18. A method of detecting the presence of a fungal infection in an individual, said method comprising:-
20
- (i) obtaining a sample from an organism; and
 - (ii) detecting the presence in the said sample of a polynucleotide or a polypeptide according to any one of claims 1 to 9, or variant thereof.
- 25 19. A method of detecting the presence of a fungal infection according to claim 18, wherein the fungal infection arises from infection with an organism independently selected from a group of genera consisting of *Aspergillus*; *Blumeria*; *Candida*; *Cryptococcus*; *Encephalitozoon*; *Fusarium*; *Leptosphaeria*; *Magnaporthe*; *Phytophthora*; *Plasmopara*; *Pneumocystis*; *Pyricularia*; *Pythium*; *Puccinia*;
30 *Rhizoctonia*; *Trichophyton*; and *Ustilago*.

20. A method of detecting the presence of a fungal infection according to claim 18 or claim 19, wherein the fungal infection arises from infection with an organism independently selected from a group of species consisting of *Aspergillus flavus*; *Aspergillus fumigatus*; *Aspergillus nidulans*; *Aspergillus niger*; *Aspergillus parasiticus*; *Aspergillus terreus*; *Blumeria graminis*; *Candida albicans*; *Candida cruzi*; *Candida glabrata*; *Candida parapsilosis*; *Candida tropicalis*; *Cryptococcus neoformans*; *Encephalitozoon cuniculi*; *Fusarium solani*; *Leptosphaeria nodorum*; *Magnaporthe grisea*; *Phytophthora capsici*; *Phytophthora infestans*; *Plasmopara viticola*; *Pneumocystis jiroveci*; *Puccinia coronata*; *Puccinia graminis*; *Pyricularia oryzae*; *Pythium ultimum*; *Rhizoctonia solani*; *Trichophyton interdigitale*; *Trichophyton rubrum*; and *Ustilago maydis*.
21. A recombinant DNA molecule comprising a polynucleotide as defined in any one of claims 1, 6 and 9, or fragment thereof, or variant thereof.
22. A recombinant DNA molecule according to claim 21, wherein the fragment is a functional fragment of the polynucleotide.
23. A recombinant DNA molecule according to claim 21 or claim 22, wherein the fragment of the polynucleotide comprises a polynucleotide sequence encoding any region of DNA shown in Table 1, or any combination thereof.
24. A recombinant DNA molecule according to any one of claims 21 to 23, wherein the polynucleotide encodes any of the motifs or regions as set out in claims 3 or 4.
25. A cell containing a recombinant DNA molecule defined by any of claims 21 to 24.
26. An organism which is transgenic for a recombinant DNA molecule as defined by any one of claims 21 to 24.

27. A cell in which a polynucleotide or polypeptide according to any one of claims 1 to 9, or variant thereof, is non-functional and/or inhibited.
28. An organism which has been genetically engineered to render a polynucleotide or polypeptide according to any one of claims 1 to 9, or variant thereof, non-functional and/or inhibited.
29. An antibody exhibiting immunospecificity for a polypeptide of any one of claims 2-5, 7 and 9, or fragment or variant thereof.
30. A method for preventing or treating a fungal infection, the method comprising administering to an individual a polynucleotide or a polypeptide according to any one of claims 1 to 9, or a variant thereof, each being optionally modified; or a modulator thereof.
31. A method of killing or impairing the growth of a fungus comprising modulating a polynucleotide or polypeptide according to any one of claims 1 to 9.
32. A method according to claim 31 wherein the fungus has infected a human or animal individual or a plant.
33. A fungus which has been killed, or whose growth has been impaired, by inhibition of the expression or activity of a protein or polynucleotide as defined in any one of claims 1 to 9.
34. A process for hydrolyzing a phospholipid or lysophospholipid, comprising treating the phospholipid or lysophospholipid with the phospholipase of any of claims 2-5, 7 or 9.
35. The process of claim 34, which is a process for improving the filterability of an aqueous solution or slurry of carbohydrate origin which contains phospholipid.

36. The process of claim 34 which is a process for reducing the content of phospholipid in an edible oil, comprising treating the oil with the phospholipase so as to hydrolyze a major part of the phospholipid, and separating an aqueous phase
5 containing the hydrolyzed phospholipid from the oil.

37. A process for the production of dough comprising adding a phospholipase according to anyone of claims 2-5, 7 or 9.

10 38. A process for the production of a baked product from a dough as prepared by the process of claim 37.

39. Use of a phospholipase according to anyone of claims 2-5, 7 or 9 for the preparation of a dough and/or the baked product thereof.

15

1/6

	1	11	21	31	41	51	61	71	81	91
PLCA			---MTVLSLA	VFTTLGFGAG	AAPHVPHGR	SSPKNVVVL	VQENLSFDNY	AGGLTYSF--		---SIDGLVNR
PLCB	-----MH	PSALLGLLAF	T-----VA	ASAIAPPAS	AHSKHSINNF	KSKJKNVVIL	VMEKRSFDNI	LGGQKLKG--		---LENPVNT
PLCC	-----MK	ANTLVPLLLGL	SSSAAAAVA	ASGNTFPFGYA	SGSKESIANL	KDKJENVVWI	LLENRSFDNI	LGGVKKRG--		---LDNVVNN
Anid1										
Anig1										
Mpor1	-----MK	ANTLIPLLLGL	SGTALGAAVT	RGNQEPWGYK	SGSKESIANL	KDKJENVVWI	LLENRGFDNI	LGGVHKEG--		---LDNVVNN
Athall			-----MSI	KAFALIQLLS	VTILYNHVA	TSPJKTIVVV	VMEKRSFDNI	LGMWKKLM--		---PEINGVDGS
Athall2			-----MVEETS	S-----GGSS	ASPKTIVVL	VQENRSFDNI	LGMWKKLM--			---PEIDGVSES
Athall3				MIETTKGSSG	SYPKTIVVL	VQENRSFDNI	LGMWKKLM--			---REIDGVTKS
Athall4				MAET-KKGSE	SYPKTIVVL	VQENRSFDNI	LGMWKKLM--			---REIDGVTKS
Athall5		-----MAFR	RVLTITVILFC	YLISSQSIE	FKNSQKPHKI	QGPJKTIVVV	VMEKRSFDNI	LGMWKKLM--		---PEIDGLTGT
Athall6										
Rice1			-----MGR	RLLLFLMLA	QAP---NSNG	DSKJKNVVVL	ALENRSFDNI	LGMWQRLLG--		---LPIDGLTGA
C_cresc		-----MPLDR	RSLLAALGAL	ALPPALARA	AIDAVRSRG	IQDVEHVVL	VQENRSFDNI	FGTLNGVRGF	GDPRFPIVRD	AAGRKSESVF
PHLA MYCTU-MSASPLLM		SRREFLTKLT	GAGAAFLMD	WAAPVIEKAY	GAGP---CPGH	LTDJEHIVVL	VQENRSFDNI	FGTLSSNGF	NA-----ASP	AFQMQGWNPM
PHLB MYCTUMGEHVPDGM		TRRQFFAKAA	AATTAGAFMS	LAGPIIEKAY	GAGP---CPGH	LTDJEHIVVL	VQENRSFDNI	FGTLSSNGF	DD-----TTPPV	FAQSGWNPM
PHLC MYCTUMVSGGAFAGM		SRRAFLAKAA	GAGAAAVLTD	WAAPVIEKAY	GAGP---CSGH	LTDJEHIVVL	VQENRSFDNI	FGTLSSNGF	DT-----PTP	LFOQKGNWPE
PHLD MYCTU-MSQSHIGGV		SRREFLAKVA	AGGAGALMS-	FAGPVIEKAY	GAGP---CSGH	LTDJEHIVVL	VQENRSFDNI	FGTLSSNGF	NT-----VSP	LFOQKGNWPM
PHLN BURPS---MTNQNR		DFLRLAAGTA	GAAALQLFPP	VIREALAIKA	NR-----RTGT	IRDVEHVVL	VQENRSFDNI	FGTLSSNGF	GD-----PRP	LTLQNGKPVF
PHLN PSEAE---MISK		SRRSFIRLAA	GTVGATVATS	MLPSSIQAAI	AIPAHRRHGN	LKDVEHVVL	VQENRSFDNI	FGTLSSNGF	GD-----RMA	IPDPGQKRVW
PLCH PSEAE---MTENWKF		RRRTFLKHGA	QAATLAGLSG	LFPETLRRAL	AVEPDRTRGT	IQDVEHVVL	VQENRSFDNI	FGTLSSNGF	ND-----PRA	LKRDQKPVW
AP_Km			-----M	RYSGTLGAL	GALALKIAGV	EADNSTSTPT	TPRTYSEL--			
AP_Af					-----MKPSV	ATLLATVSLV	YAQTATEK--			
AP_Pc					-----MLTKQTL	LAFVGAALALA	TGTTTTTE--			
AP_An					-----MTFKQSL	VTLGLGLSLA	VAQTTEG--			

Box II

	101	111	121	131	141	151	161	171	181	191
PLCA	T-YCNPANIS	DPHSP-MVCA	QP-TAKNIAP	DDPDHSITGG	NQOVYSTYHE	DSD-----FD	APNQGFVAE	QIAAYGIDGN	LSRAGEVIN	YTPPEHIVPF
PLCB	GPYCNFYNL	DPHSP-MVCA	AAKDFDSITN	DDPDHAIYGN	NIEFYGTFFNE	DWTQIAEGKL	VPHNQGFVHE	QVRKYGAKVN	KTVLATQVMN	YTTREQVVPV
PLCC	GPFCNPQNV	DPHSP-MVCA	VYKDFDSVKN	DDPDHAIYGN	NIEFYGTFFNE	SNDALANGTL	KPTMNGFVEQ	QVHVKHG-LD	PKVADEEVMG	YYSDEIPTL
Anid1										
Anig1										
Mpor1	GPFCNPESVN	QTHSK-KWCS	TFKDYDSVIH	DDPDHAIYGN	NIEFYGTFFNE	SNKDIASGKL	KPNLNGFVER	QMDSYPA-IS	AKRATHEVMA	Y-----
Athall	E---SNPVSVS	DPHSP-MVCA	GS-GSHYVDP	DPGHSTFQAI	REQVFG---S	NDTSMDDPPP	---MNGFVQ	AYSEDDPS--	GNMSASVMN	GFEPPKVPVY
Athall2	EPHSPNPLSTS	DPHSP-MVCA	GK-ESQNDIP	DPGHSTFQAI	REQVFG---S	DESYPDPDK-	---MNGFVQ	AEAIT-K---	GMSHVMQ	GFPPEKLPVF
Athall3	DPKSNVTVSS	DPHSP-MVCA	GD-QSQYVNP	DPGHSTFQAI	REQVFG---S	SGKPDNPNGH	PN-MSGFAQN	AERNKK---	G-MSSAVMN	GFKPNALPVY
Athall4	DQKFNPGFS	DPHSP-MVCA	GD-QSQYVNP	DPGHSTFQAI	REQVFG---S	SGKPDNPNGH	PN-MSGFAQN	AERNKK---	G-MSSAVMN	GFKPNALPVY
Athall5	E---SNPLNVS	DPHSP-MVCA	SD-DAVFDM	DPGHSTFQAI	REQVFG---S	SGKPDNPNGH	PN-MSGFAQN	AERNKK---	G-MSSAVMN	GFKPNALPVY
Athall6	E---CNPVFNS	DPHSP-MVCA	FTSDAEFVDP	DPGHSTFQAI	REQVFG---S	SGKPDNPNGH	PN-MSGFAQN	AERNKK---	G-MSSAVMN	GFKPNALPVY
Rice1	E---CNPAFGP	GPADS-LLHC	VSPADLVVP	DDPAHAFEDV	LEQLLGFRRN	DSTGAASPS	D---MSGFVRS	AVSVS-----	ALLTHAVMR	GFTPSRLPAF
C_cresc	VOAWSKDKLL	-APFP-LNSA	-QTFAHMRV	EGTPHSTWDA	QADWDQGRMD	RWPDA-----			---KKEWSMG	YFQADIPFO
PHLA MYCTUTQALDPAGVT	-IPFR-LDTT	RGPFLLDGECV	NDEPHQWVGM	HLAWNGGAND	NWLPQAQAT--			---T-RA	GP-VYELTMG	YTRQDIPFH
PHLB MYCTUTQAVDPAGVT	-LPYR-FDIT	RGPLVAGECV	NDDPDHWMGM	HLAWNGGAND	NWLPQAQAT--			---F-SF	LQGNVHTVMG	YTRQDIPFH
PHLC MYCTUTQALDPTGIT	-LPYR-INTT	GGPNGVGEVC	NDDPDHWMGM	HLAWNGGAND	NWLPQAQAT--			---T-RS	VA-NTHVVMG	YTRQDIPFH
PHLD MYCTUTQALDPTGIT	-MPYR-FDIT	RGPFLLDGACV	NDDPDHWMGM	HLAWNGGAND	NWLPQAQAT--			---T-RS	AA-HTHVVMG	YTRQDIPFH
PHLN BURPSHQPVLGPAP	LLPFH-PDAS	---NLGMOFL	QDLPHGWQDT	HGAWNGGAND	RWIAKNG---			---T-TMA	YLERQDIPFH	
PHLN PSEAEHQKSGKEI	-LPYH-FDTS	---TTSQQRV	DGTPTWTPDA	QQAWEGRMD	KWLPQA-----			---KTERSLG	YKRGDIPFH	
PLCH PSEAEHQKSGKEI	---PYH-WDTK	---VTSQQRV	SSQNHEWSAF	HAIWNGGAND	KWMAVQY---			---HEAMG	YKRGDIPFH	
AP_Km	---DPSLSEI	QAASASAVTA	HRTSDVKGA					---VNR	FVYFIENEDT	YDKAAREDES
AP_Af	---EPSLSAI	ESAAASIQPY	SPVSNVEGVA					---FNR	FFQVWLENID	YEDAAADENM
AP_Pc	---TPTQAEI	DAARATAPY	SPVSNVKGVA					---FDR	FVNIWLENID	FEPALDENL
AP_An	---YPSLAET	RAAQATVQPY	SPVSNVKGVA					---FNR	FVNIWLENID	FDAAAATEHL

Box II cont.

Box III

	201	211	221	231	241	251	261	271	281	291
PLCA	NAMAEVFLV	DRWFASVPP	TNPNRAYLTS	GTSHGHGTND	-----	---PDFDRSA	LQPSIFEQ	SEHNISWNY	SWTT-----	
PLCB	TSLVQNYLTF	NHWHSDIPGN	TNPNRALVS	GTSLGHGLND	-----	---AAFTKHE	FPQSFIFQQL	TETGHSWNY	VDPA-----	
PLCC	VNLVDEFTTF	NHWHSCVPGP	TNPNRALVS	GTSDGHGTND	-----	---NSFDVSG	VDISIFQVA	SEKDTWKYJ	LGTN-----	
Anid1										
Anig1										
Mpor1										
Athall	KSLVSEFAVF	DRWFASVPP	TQPNRMFVHS	GTSAGATSNM	-----	---PISLAKG	YPOFTIFDNL	DDEEFSFGIY	YQN-----	
Athall2	KELVQFAVC	DRWFASVPP	TQPNRMFVHS	ATSHGATSNM	-----	---TNTLVRG	FPQFTVFESL	EESGFTFGIY	YQS-----	
Athall3	KELVQFAVC	DRWFASVPP	TQPNRMFVHS	ATSHGATSNM	-----	---KKLLLEG	FPQFTVFESL	DEAGFTFGIY	YQF-----	
Athall4	KELVQFAVC	DRWFASVPP	TQPNRMFVHS	ATSHGATSNM	-----	---KKLLLEG	FPQFTVFESL	DEAGFTFGIY	YQF-----	
Athall5	TELANERGVF	DRWFASVPP	TQPNRMFVHS	ATSHGATSNM	-----	---KKDLVKG	FPQFTVFESL	DENGFTFGIY	YQN-----	
Athall6	AELVKEFAVF	DRWFASVPP	TQPNRMFVHS	ATSHGATSNM	-----	---KKDLVKG	FPQFTVFESL	DENGFTFGIY	YQN-----	
Rice1	SALASSFAVF	DRWFASVPP	TQPNRMFVHS	ATSHGATSNM	-----	---KWNLLRG	YPOFTIFDNL	AADALDYRVY	EKT-----	
C_cresc	FALADAFLLC	DAYHCSITQT	TNPNRALVS	GTNDGLGKAG	GPSISNGHND	FAEKGKAKES	YTWITYPERL	LQAGVSWRIY	CD-----	
PHLA MYCTUTQALDPTGIT	DAYHCSITQT	DAYHCSITQT	TNPNRALVS	GTNDGLGKAG	GPSISNGHND	FAEKGKAKES	YTWITYPERL	LQAGVSWRIY	CD-----	
PHLB MYCTUTQALDPTGIT	DAYHCSITQT	DAYHCSITQT	TNPNRALVS	GTNDGLGKAG	GPSISNGHND	FAEKGKAKES	YTWITYPERL	LQAGVSWRIY	CD-----	
PHLC MYCTUTQALDPTGIT	DAYHCSITQT	DAYHCSITQT	TNPNRALVS	GTNDGLGKAG	GPSISNGHND	FAEKGKAKES	YTWITYPERL	LQAGVSWRIY	CD-----	
PHLD MYCTUTQALDPTGIT	DAYHCSITQT	DAYHCSITQT	TNPNRALVS	GTNDGLGKAG	GPSISNGHND	FAEKGKAKES	YTWITYPERL	LQAGVSWRIY	CD-----	
PHLN BURPSHQPVLGPAP	DAYHCSITQT	DAYHCSITQT	TNPNRALVS	GTNDGLGKAG	GPSISNGHND	FAEKGKAKES	YTWITYPERL	LQAGVSWRIY	CD-----	
PHLN PSEAEHQKSGKEI	DAYHCSITQT	DAYHCSITQT	TNPNRALVS	GTNDGLGKAG	GPSISNGHND	FAEKGKAKES	YTWITYPERL	LQAGVSWRIY	CD-----	
PLCH PSEAEHQKSGKEI	DAYHCSITQT	DAYHCSITQT	TNPNRALVS	GTNDGLGKAG	GPSISNGHND	FAEKGKAKES	YTWITYPERL	LQAGVSWRIY	CD-----	
AP_Km	AWLAKQGISL	TNYW-SLTHP	SEPNYLASVA	-GDYFALDND	-----	---REISMP	SNVSNIVDNL	DTKNISWAEY	CEHIPYTGFO	GFNYSNQETF
AP_Af	KWLASQGISL	TNYW-SLTHP	SEPNYLASVA	-GDYFALDND	-----	---REISMP	SNVSNIVDNL	DTKNISWAEY	CEHIPYTGFO	GFNYSNQETF
AP_Pc	SKLAKQGISL	TNYW-SLTHP	SEPNYLASVA	-GDYFALDND	-----	---REISMP	SNVSNIVDNL	DTKNISWAEY	CEHIPYTGFO	GFNYSNQETF
AP_An	PVLAKQGISL	TNYW-SLTHP	SEPNYLASVA	-GDYFALDND	-----	---REISMP	SNVSNIVDNL	DTKNISWAEY	CEHIPYTGFO	GFNYSNQETF

Figure 1

Box X

	501	511	521	531	541	551	561	571	581	591
PLCA	--GN-GFETH	TSILKVFADL	WGLEY----	--LSPRVAVS	ASFAHLIT--	--DTFREDTPA	TLPEPADF--	-----	-----	-----
PLCB	HGET-VSYSA	ASILRLTGLY	WDFEP----	--TPRVEHA	ASFEHLIR--	--LHARKDTP	ALPSAVPFRN	RNV-----	-----	-----
PLCC	ATGKSASYSA	TSVLKLTGLD	WDLDD----	--FTPRVSHS	PAFDHLIG--	--PKK-RNTPA	TLANPHFPFD	AV-----	-----	-----
Anid1	-----GYTH	TSILKVDVEL	WGLTI----	--LTPREVVS	ASFGDLIE--	--KKFREDTPE	LLPEPVIF--	-----	-----	-----
Anig1	--QP-NDFTH	TSILKYLSEL	WDLFV----	--LTPRVAVS	PSFGGLIT--	--TTYRDDTDP	KLPEPVDF--	-----	-----	-----
Mpor1	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

Athal1	PFFS-SBEYH	SSIPATVKKL	FNLSSP----	--FLTKRDEWA	GTFENILQ-I	RKEPRTDCPE	TLPEPVKIR--	----MGEANE	KA--LLTEFQ	-----
Athal2	PEPT-SQFEH	SSIPATLKKI	FNLS-----	--FLTKRDEWA	GTLDAVIN--	RTSPRTDCPV	TLPELPFRARD	--IDIGTOEE	DE--DLTDFQ	-----
Athal3	YPFR-SQYEH	SSIPATVKTI	FKLKD----	--FLSKRDSWA	GTFESVIT--	RDSPRQDCPE	TLSTPIKLRG	----TMAKE	NA--QLSEFQ	-----
Athal4	PYLM-SQYEH	SSIPATVKFI	FKLKD----	--FLTKRDSWA	GTFESVIT--	RNSPRQDCPE	TLSNPVKMRG	----TVAKE	NA--ELSDFQ	-----
Athal5	PTPH-SQFEH	SSIPATVKKL	FNLS-----	--HFLTKRDEWA	GTFEKYFR-I	RDSPRQDCPE	KLPEVKLSLR	----PWGAKE	DS--KLSEFQ	-----
Athal6	PTES-SBEYH	SSIPATIKKL	FNLS-----	--NFLTHRDWA	ATFEDVVS-H	LTPRTDCPM	TLPEVAP-MR	----ATEPKE	DA--ALSEFQ	-----
Rice1	PTPT-SBEYH	SSIPATIKKI	FNLSDD----	--FLTRRDWA	GTFEHLFT-D	LDEPRTDCPE	TLPEIIPPPS	----SSSTKKE	DGG-WLSDFQ	-----

C_cresc	-----QVFDH	TSVLRFLQOR	FGVMEP----	--NISPWRRAVC	GDLTTCFD-F	KTPNTNPFPA	LPATRE-----	---PAGRAAR	LGRAPPTTPA	-----
PHLA MYCTU	-----DFDFH	TSQLKLIRAR	FGVVEP----	--NMTAWRDGVV	GDMTSAFN-F	ATPPNSTRRP-	-----	---NLSHF-LLGA	LPKLQPCIPN	-----
PHLB MYCTU	-----DFDFH	TSYTLKLIRAR	FGVVEP----	--NLTAWRDGVV	GDMTSTFN-F	A AAPNPSKPF-	-----	---NLDHP-RLMA	LPKLQPCVFN	-----
PHLC MYCTU	-----DRFDH	TSQLQLIGKR	FGVVEP----	--NLTPWRASVT	GDMTSAFN-F	A AAPDPSPFP-	-----	---NLDHP--VRQ	LPKVAKCVFN	-----
PHLD MYCTU	-----DFDFH	TSQLRLLETR	FGVVEP----	--NLTAWRASVT	GDMTSTFN-F	A AVPPNSGSPW-	-----	---NLDPY-GLHA	LSTVPQCVPN	-----
PHLN BURPS	-----QVFDH	TSLLQFIEAR	FNDYVPVRAP	NVTPWRRRAVC	GDLTSAFN-F	SSPDG-SWQP	LPDTSGY--	--APPD-RNRH	P-SYVPEVPV	-----
PHLN_PSEAE	-----QVFDH	TSVIRFLQOR	FGVMEP----	--NISPWRRRAVC	GDLTSAFN-F	ANPNNEPTEF	LPDTS-----	---QADAIVASQ	IKLPPKPPPA	-----
PHLN_PSEAE	-----QVFDH	TSVLRFLERR	FGVVEE----	--NISPWRRRAVC	GDLTSLFD-F	QGAGDTQVAP	DLTNVPQS-A	RKEDAYWQQF	YRP-SPKYWS	-----

AP_Km	GTSDDTTYTH	YSQLATVEAN	WNLEH----	--LGRHVDV	ANVLQIVANA	TNITNVEVDT	RYMLNNETYI	GYLNDG----	TLPIPLPNVT	-----
AP_Af	GTQDDTTYTH	YSVIAVSAN	WGLHS-----	--LGRWDGC	ANILEIVANK	TGYNVYDVT	TNLRNLETYP	GPMSAG----	EYSKYSPPWA	-----
AP_Pc	GTDDTTYTYH	YSIVASLSAN	WGLHS-----	--LGRWDGC	ANLLKMVADK	TGYNVYDVT	SNVLYNETYP	GPMSDT----	NYSSK--WGA	-----
AP_An	GTKDDTTYTH	YSVIAFSLTN	WGLHS-----	--LGRWDGC	ANLFSWLAKK	TGYNVYDVT	SNLYMNETYP	GPT.SDN----	DYSEYAGGWR	-----

Sequences continue

Figure 1 (continued)

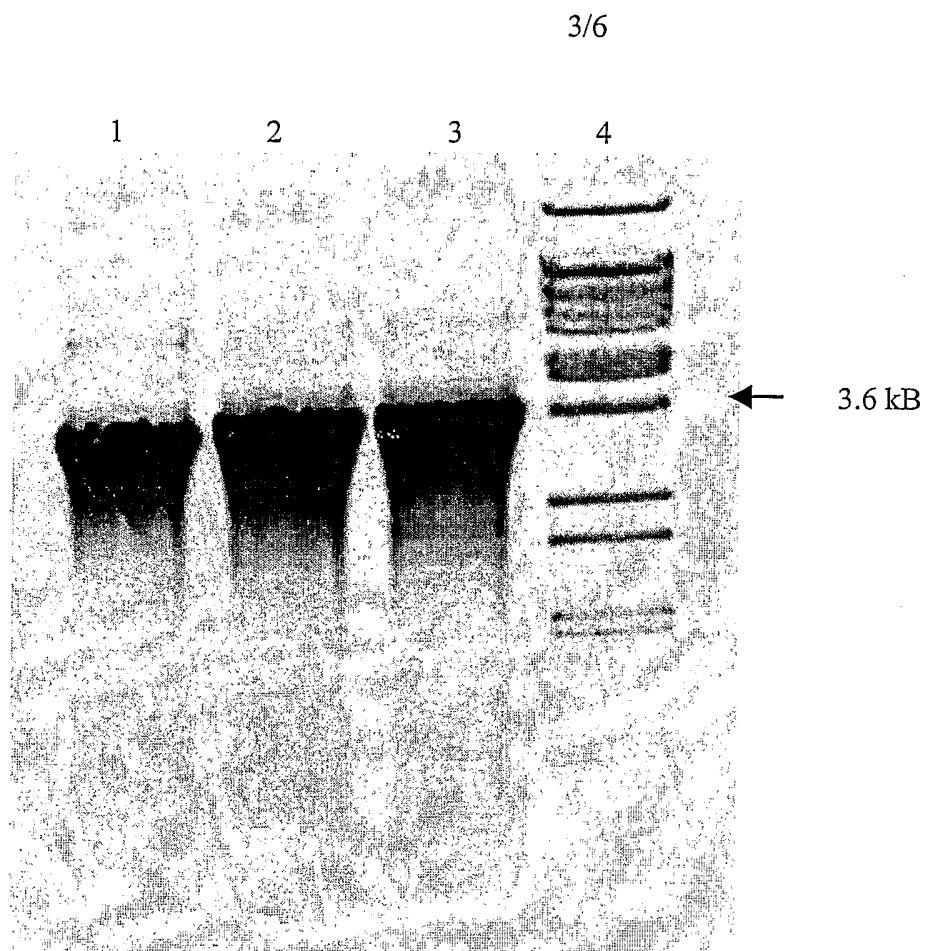


Figure 2.

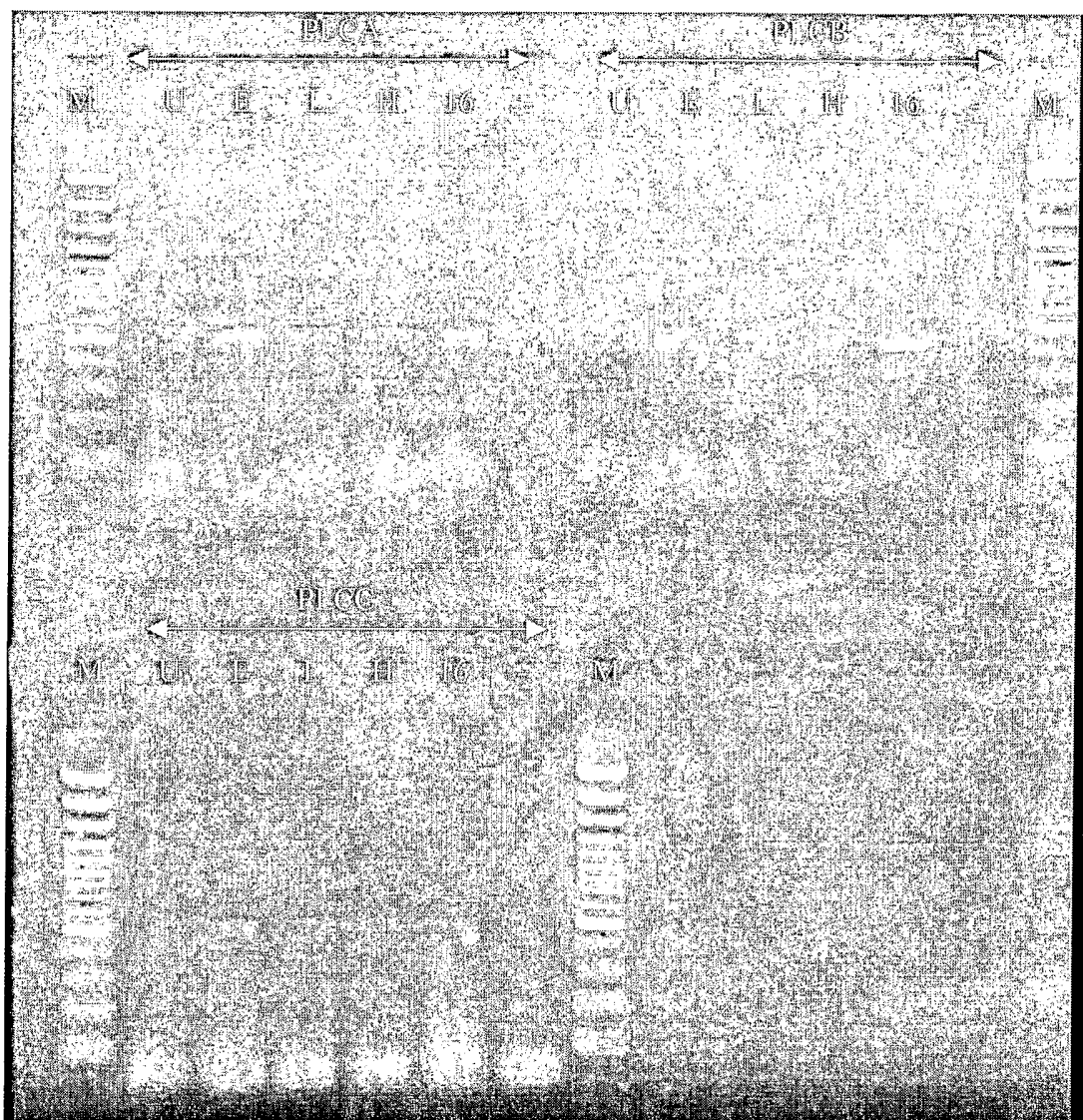


Figure 3.

5/6

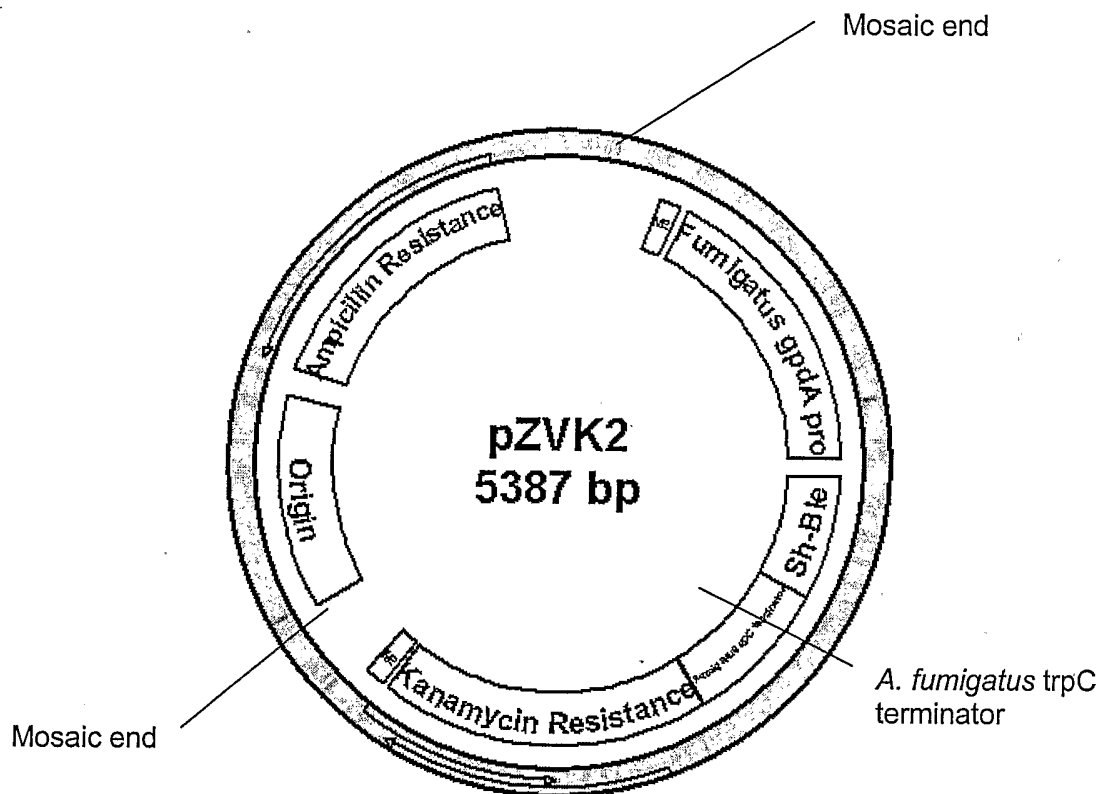


Figure 4.

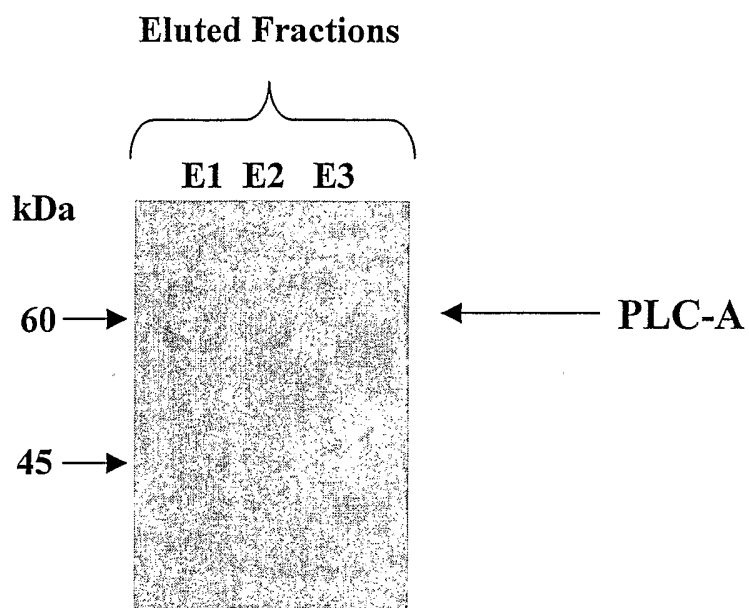


Figure 5

1

SEQUENCE LISTING

<110> F2G Ltd.<120> PHOSPHOLIPASE C<130> AH/Y771<160> 84 <170>
 PatentIn version 3.0<210> 1<211> 3490<212> DNA<213> Aspergillus
 fumigatus<400> 1

```

ccattatgcc tggagtcgtt tgtctttgct tggctctgca gtgggggtggc tcccagtatg      60
cagtacgttc tcattcatca ctggctttgt tcaccaactgg tattgactgc gtctagtggg      120
gagagggccg gatcgctcgc ctgctgggtgc tgaccttcct gtgtctgata gcgttcgtct      180
tgatccaggt atggaagcca gagcaggcaa cgctgccacc acgagtcttc ctacaacgga      240
gcatcgcttc cggttttctgg gcgagctgct gtttcggcgc acacatgata gtgttcgggtg      300
agtcggctgg atttcaacct ccaactgatgg gatccgcctg ctaatagcca accgtgattt      360
atcttcccct ctggttccag gtcattgacg gcgtctcggc cgtcgacagc ggcacccgcc      420
tcctcccgat gctgctgtca ctggtggtag cgtccattct cactggcggg ctagtctccc      480
gcatcggtta ctatacgccc tttctgatgc tcggcatcgt cctctcctcc atcggcgccg      540
gtctgctcac cactctcggg gtccatacgt caagcgccaa atggattggt tttcagggtgc      600
tgtatggttt tggcctcggc acctgcaacc agataccaaa catggcggcg cagacagtgc      660
tcccccgaga gcaggtggcc atcggcgcgct cgctcatgtt ctttgcgcaa cagctgtttg      720
gogccgtctt tacgtcggtc ggcgagaacg tgctcagcaa ccagctggcc aagcgtctgg      780
cgatcagttc gcaaacggtc cagaataacc gtgccacca gcttctggag cacgttcctg      840
tcgacgaccg tgttgcatcg ctgggggctt ataatgactc gctgcaggta gttttccagg      900
ttggtctcat catggcttgt cttgccatcc tgggtgcgtt acctatggaa tggcgcaactg      960
tcaagaagga gcagctgtct tccacgtctg accgcaacga tggacaggcg gaggagggcg      1020
ccccgggcaa tgcccagtca acgatggact acaagcaggg gacggtgaat tgaatgtaac      1080
ggaatctaga tgcatcataa tcggtgcaga actgtacaat agtgaataga ggcatatcag      1140
caaatggaga gatttgttcc aagactccaa gtgcgggaga catccgcaaa tcagcgccac      1200
aaagtgacaa gccaatagaa tcttcattcc ccagccacg cgccggttat tctogtgaag      1260
acaggctctc gcactcccag gcactagggc tgctgtgccc aataagatgc agtatcgccc      1320
acatgttgac gggcgagtgg atgccgatg atcagatcag gatcagcagc caagatcaac      1380
cccacgtccg ggagggagga tgatcttcgt ggatgacggg cgagttattc tcacgattct      1440
tatcaacatc ccctgaaagg gttacagaac cactgtcaaa atgcaacca aagcagttca      1500
agatgcacat gcctcatttc cggcagggaa acatgcgcg gacgagacgg agcatctgct      1560
gccgcatgat ccccagcgg agataaatat ctgggggtcg cgcagacgac cccgttcctg      1620
gtgggctctc gtcgccatct cgtgctgct gttgaccatg actgtcctgt cgctggctgt      1680

```

tttctttcacg ctgggctttg ggcgaggcgc ggcgcctcat gtgccccacg gcagactgtc 1740
cagtccgatac aagaatgtcg tggttctggg gcaggagaac ctgtcgttcg acaactatgc 1800
cggcggcctc acatatagcc ccagtattga cgggcttgtg aaccgcacgt actgcaaccc 1860
ggccaatatc tccgatcccc actcgccaat ggtgtgcgca cagcccacgg cgaagaacat 1920
cgccccggat gatccggacc acagtatcac cggcggaat cagcaagtgt acagcaccta 1980
ccatccagac tccgacttcg acgccccaa catgcaaggc ttctgtcggg agcagatgc 2040
cgcatacggc atcgacggca acctctcgcg cgccggcgag gtcataact actatacccc 2100
cgagcacatc cccgtcttca acgccaatggc cgagaacttt gtgctcttcg accgctgggt 2160
cgctgctgtt cccggcccaa ccaaccccaa ccgcgcctat ctgacgtccg gcaacttcca 2220
cggccacggc accaacgacc cggacttcga caggctcggc cccccacg gttccatctt 2280
cgagcagctc tccgaacaca acatcagctg gatcaactac tccaacacga cgggttct 2340
ccccgacgcg ctcttctacc gctggaccgc cgtgtcgggt ctggcgaga ccaatgtccg 2400
ccccatcgac cagttctacc gcgacgcaa agcaggcacc ctgccccagt tcacctggat 2460
caaccggaa tgctgctcgt acatgtcctt ccaccgcgcg tcgcccatac atatgggcga 2520
gggctggatc aagagcgtct acgaggcgt cgcctcgtcc ccgcagtggc acgagactct 2580
cttcatcctg acctttgacg agcacggcgg gttcgcggac cactgcccgc ccccgagaa 2640
cgtccctgcg ggcgatgatac taacctacac cgagacggcg cgggacggaa agccctccac 2700
ctttgcgttt gaccgggttg ggatccgggt gccgacggtg ctgatgtcgc cgtgggtggg 2760
taaggggggt gtgcagaacc gaccagcaga tgggaacggc gagttcacgc atacgtctat 2820
cctgaagttt gtggctgatac tgtgggggtt ggagtaacctg tcgcccggg tggcctggtc 2880
tgcgagtttt gcgcatttga ttacggatac gtttcgggag gatacgccg caacgttacc 2940
tgagccagcg gattttotaga tgtagtcaga tctgataga tgtttttgtc ttacaagggt 3000
tagcattaca cggagagctg aatgaatacg tctcgtacgg ctgcattaag tagcggagct 3060
atacaggta tatgcctatc aagtcttgac tgcagctagg aactcgtgtg gccatttcga 3120
tctcctagtg aacctataa ggtaatagac acatggtaat agcatacacc tggctgtacg 3180
gggggtgagt tgaataacag cggatggaag cctcacgttt taacacatac accgcaacat 3240
tgaggcctct ggatatcaga caaaaagtgc ttctcggcgt tattcgggtg ttcggacgag 3300
ggcttataac cgggtcggag tgggcacaag cattccagac gctcatccct cgctcgcag 3360
gaatgtcacc gactgcatat gcgcgaagag cggatacaac tggtagggtt gtacttgctt 3420

3

cgaagataga aataagatct gtgatatctg ttttggcaat tggaggccgt ttctattcac 3480
ggctcaggct 3490

<210> 2<211> 1302<212> DNA<213> Aspergillus fumigatus<400> 2
atgactgtcc tgtcgtggc tgttttcttc acgctgggct ttggcgcagg cgcggcgcct 60
catgtgcccc acggcagact gtccagtccg atcaagaatg tcgtggttct ggtgcaggag 120
aacctgtcgt tcgacaacta tgccggcggc ctacatatata gcccagtat tgacgggctt 180
gtgaaccgca cgtactgcaa cccggccaat atctccgata ccactcgc aatgggtgtgc 240
gcacagccca cggcgaagaa catcgccccg gatgatccgg accacagtat caccggcggg 300
aatcagcaag tgtacagcac ctaccatcca gactccgact tcgacgcccc caacatgcaa 360
ggcttcgtcg cggagcagat cgcgcatac ggcatcgacg gcaacctctc gcgcgccggc 420
gaggtcatca actactatac ccccgagcac atccccgtct tcaacgcat ggccgagaac 480
tttgtgctct tcgaccgtg gtctcgtgct gtcccgggc caaccaaccc caaccgcgcc 540
tatctgacgt ccggcacttc ccacggccac ggcaccaacg acccgactt cgacaggtcg 600
gcgctcccc agcgttccat cttcgagcag ctctccgaac acaacatcag ctggatcaac 660
tactccaaca cgaccggctt cctccccgac gcgctcttct accgctggac cgccgtgtcg 720
ggtctcggcg agaccaatgt ccgccccatc gaccagttct accgcgacgc caaagcaggc 780
accctgcccc agttcacctg gatcaaccg gaatgctgct cgtacatgtc cttccaccg 840
ccgtcgccca tcaatatggg cgagggctgg atcaagagcg totacgaggc gctccgctcg 900
tccccgcagt ggcacgagac tctcttcac ctgaccttg acgagcacgg cgggttcgag 960
gaccacgtgc cgcgcccgga gaacgtccct gcgggcgatg atctaacct caccgagacg 1020
gcgcgggacg gaaagccctc cacctttgag tttgaccggt tggggatccg ggtgccgacg 1080
gtgctgatgt cgccgtgggt gggtaagggg gtggtgcaga accgaccagc agatgggaac 1140
ggcgagttca cgcatacgtc taccctgaag tttgtggctg atctgtgggg gttggagtac 1200
ctgtcgccgc ggggtggcctg gtctgcgagt tttgcgcatt tgattacgga tacgtttcgg 1260
gaggatacgc cggcaacgtt acctgagcca gcggatttct ag 1302

<210> 3<211> 433<212> PRT<213> Aspergillus fumigatus<400> 3

Met Thr Val Leu Ser Leu Ala Val Phe Phe Thr Leu Gly Phe Gly Ala
1 5 10 15

Gly Ala Ala Pro His Val Pro His Gly Arg Leu Ser Ser Pro Ile Lys
20 25 30

4

Asn Val Val Val Leu Val Gln Glu Asn Leu Ser Phe Asp Asn Tyr Ala
 35 40 45
 Gly Gly Leu Thr Tyr Ser Pro Ser Ile Asp Gly Leu Val Asn Arg Thr
 50 55 60
 Tyr Cys Asn Pro Ala Asn Ile Ser Asp Pro His Ser Pro Met Val Cys
 65 70 75 80
 Ala Gln Pro Thr Ala Lys Asn Ile Ala Pro Asp Asp Pro Asp His Ser
 85 90 95
 Ile Thr Gly Gly Asn Gln Gln Val Tyr Ser Thr Tyr His Pro Asp Ser
 100 105 110
 Asp Phe Asp Ala Pro Asn Met Gln Gly Phe Val Ala Glu Gln Ile Ala
 115 120 125
 Ala Tyr Gly Ile Asp Gly Asn Leu Ser Arg Ala Gly Glu Val Ile Asn
 130 135 140
 Tyr Tyr Thr Pro Glu His Ile Pro Val Phe Asn Ala Met Ala Glu Asn
 145 150 155 160
 Phe Val Leu Phe Asp Arg Trp Phe Ala Ala Val Pro Gly Pro Thr Asn
 165 170 175
 Pro Asn Arg Ala Tyr Leu Thr Ser Gly Thr Ser His Gly His Gly Thr
 180 185 190
 Asn Asp Pro Asp Phe Asp Arg Ser Ala Leu Pro Gln Arg Ser Ile Phe
 195 200 205
 Glu Gln Leu Ser Glu His Asn Ile Ser Trp Ile Asn Tyr Ser Asn Thr
 210 215 220
 Thr Gly Phe Leu Pro Asp Ala Leu Phe Tyr Arg Trp Thr Ala Val Ser
 225 230 235 240
 Gly Leu Gly Glu Thr Asn Val Arg Pro Ile Asp Gln Phe Tyr Arg Asp
 245 250 255
 Ala Lys Ala Gly Thr Leu Pro Gln Phe Thr Trp Ile Asn Pro Glu Cys
 260 265 270
 Cys Ser Tyr Met Ser Phe His Pro Pro Ser Pro Ile Asn Met Gly Glu
 275 280 285
 Gly Trp Ile Lys Ser Val Tyr Glu Ala Leu Arg Ser Ser Pro Gln Trp
 290 295 300
 His Glu Thr Leu Phe Ile Leu Thr Phe Asp Glu His Gly Gly Phe Ala
 305 310 315 320
 Asp His Val Pro Pro Glu Asn Val Pro Ala Gly Asp Asp Leu Thr
 325 330 335
 Tyr Thr Glu Thr Ala Arg Asp Gly Lys Pro Ser Thr Phe Ala Phe Asp
 340 345 350

Arg Leu Gly Ile Arg Val Pro Thr Val Leu Met Ser Pro Trp Val Gly
355 360 365

Lys Gly Val Val Gln Asn Arg Pro Ala Asp Gly Asn Gly Glu Phe Thr
370 375 380

His Thr Ser Ile Leu Lys Phe Val Ala Asp Leu Trp Gly Leu Glu Tyr
385 390 395 400

Leu Ser Pro Arg Val Ala Trp Ser Ala Ser Phe Ala His Leu Ile Thr
405 410 415

Asp Thr Phe Arg Glu Asp Thr Pro Ala Thr Leu Pro Glu Pro Ala Asp
420 425 430

Phe

<210> 4<211> 3159<212> DNA<213> Aspergillus fumigatus<400> 4
atcatcaacc tgcgtattcc cgagcttcgt cgacgcggat tgttctggga gaattatgct 60
gtcgcagaggg gaacgtatcg ggagaatctg tatggcaagc ctgggcagac aggtcccccg 120
gcggatcatc ctgcgtcgag gtaccggtgg catgcagggg tagacgctgc cgatcataag 180
attcccgaaa actagtacga ccgacgcgca gtgacttcgg cctagcagcg gccgtagact 240
gtcccgactc gagcgtcgag ttgaatctct atggtagtgg gaaatagttc cagcaggaca 300
gtcgtgcgca tctaccgtaa tctgtactag agagggtgaa cacctatctg taggaccaat 360
tccaaaocca gacgattgat tgtcgtatcg gacaagaaag ctctcgactt ttcctaaat 420
ctgaatgcag catgcacaac aatatggcaa ctgcttgctc ttcgtcactc cgtcgattct 480
gcgtacatcc ttgacagact ggtcttctag aacctgtagc gacatgaggt tatgccgata 540
gcattatcaa caacagagta actcacgagg ctgcatgggt atgcaagata tcctgcagat 600
attgccgtat tgccgtcttc caatgattgc agagagatgc agcacgggga aggcgttcga 660
gggttttcg ctttacttc tcgtgttcct ggccgcta at tggcatctag tgaggggcac 720
accacgatg aagccgtctt ggcaaacatt cgggcgtcga atcggttgcg tgttgaccca 780
gcttgacgat tcttggtgtt gacattggct gcttggcca cgccttgctc agaaggcacc 840
cctgctaagt gatcttttgt gtcttttctc tgccatgttc aaggacaga gctccattcg 900
agcacgacag cacggatgtg gcttggcctt gcataatttc cccgcctgca gccaagcggc 960
aaaccctgt actgtccagg tgctctcgtc gcctcgtgct tctgaagatt cattttattg 1020
gagaagggtg caggatgtgt cggctcttcc cgccacgcct gttgagaact cattttccga 1080
catcttgcta aggaacatct ccaactatac cattcgttcg cttgggcata gtttacacag 1140
agaagatgca acgcaatccg tgtctccaga gcacgtgggg gaggactgaa gggccaggac 1200

gatgctgagg atttcgagga ctggtataaa atgggcttcc gcaaccggtc gacaggatgt	1260
tccctgaaaa gcagcatcct ctgaccagtc ttctctgctt catctctgca gaatgcatcc	1320
tagtgctctt cttgggtctcc tggccttcac ggtcgccgcc tcggccattc ctgcaccgcc	1380
agccagtgcg cactccaaac actcaatcaa caatttcaag tcgaagatca agaattgtgt	1440
catcctgggtg atggaaaacc gtccttttga caacctcctc ggtgggtcaga agctcaagggt	1500
cctggagaac cccgtcaaca ccggccctta ctgcaacccc tacaacctca ccgaccccag	1560
ccaaggcggtt gcctgttctg ccgccaagga cttcgactcc atcaccaatg accccgacca	1620
cgccatctac ggcaacaaca ttgagttcta tggcaogttc aaccagaca aactcagat	1680
cgccgagggc aagctggttc ctcataacca aggcttcggt catgagcagg tccgcaagta	1740
cggcgccaag gtcaacaaga ccgtcctcgc taccaggtc atgaactact acacogaaga	1800
gcagggtcca gttctgacct cgctggtgca aaactacctg accttcaacc actggcactc	1860
ggatatcctt ggcgtgagta tcttatgcct ttgaccttg caggaagctg actgactctg	1920
cagaacacca accccaaccg tgccgccctg gtttctggta cctccctcgg ccacgggtctg	1980
aacgacgctg cttttaccaa gcacgagttc cccagcggt ccatcttcca gcagttgacc	2040
gagaccggtc actcgtggat caactatgtc gacctgcgg gcggtaccgg cctgatgca	2100
ggctacttca actggacctt tgctaccaac aaccaagaca agatccagcc cctggccaag	2160
ttctacactg acgctgcggc tgggtgccctt ccogaattgg cgtaccttaa tccctcgtgc	2220
tgcgaggttg gcaccacctc catgcatcct gocggcctga tctccgatgg cgaggctttc	2280
atcaagaagg totacgatgc tctgcgcgcc ggtccccagt gggaggagac cctcttcac	2340
ctgaccttcg acgagagcgg tggcttccac gaccacgtct ccgctcctct cgctccccgg	2400
ccggataacc tgacctttac totcagcacc cccagtggcg aagactacac cttccccctt	2460
gaccgtctgg gtggtcgcat cccgacctg ctgatctccc cctgggttgc caaggcacag	2520
gtcgagcaga agggtaagaa tgctcacggc gagactgtgt cctactcggc tgcacgatt	2580
ctgcggactc tgggctacct ctgggacttt gagcccttta cccctcgcgt cgagcatgcg	2640
gcgtcathtt agcaacttgat tcgtctccat gctcggaagg aactcccac tgcgtccct	2700
tctgcagtgc ctttcaggaa tagaaacgtg tagacatgga actaaatatg ggacatggta	2760
cgatcaatgc atgacaaaca atagcataga tgtttgatcc gaacagtga tccgattttc	2820
ttcaccgacc cgggtgattta cgacggacta tagctccgat gcggactttg ggtgttctca	2880
ttgatacttc gatttctggt cgtattggat ataccatttg aaggacaaca gcaacaatac	2940

7

aagcttaacc aaggtagtaa tgcaaccatc agtggttcacg ccatcgcgcc ccaaattctgg 3000
 agcagatggt cgacaatgac gtcgaaatct ctgtgaacgt cgtacgcacg tcgggtcgga 3060
 tgctcaacat agcaaaaata gtcctcctcg tccagtctgt agaaacgtac cagcggacca 3120
 actgcaatag ccgcaaaaag acttgcccga ggctttccc 3159

<210> 5<211> 1371<212> DNA<213> Aspergillus fumigatus<400> 5
 atgcatccta gtgctcttct tggctcctcg gccttcacgg tcgcccgcctc ggccattcct 60
 gcaccgccag ccagtgcgca ctccaaacac tcaatcaaca atttcaagtc gaagatcaag 120
 aatgtggtca tcctggtgat ggaaaaccgc tcctttgaca acctcctcgg tggtcagaag 180
 ctcaaggggc tggagaacct cgtcaacacc ggcccttact gcaaccctca caacctcacc 240
 gaccccgacc aaggcgttgc ctggttctgcc gccaaaggact tcgactccat caccaatgac 300
 cccgaccacg ccatctacgg caacaacatt gagttctatg gcacgttcaa ccagacaac 360
 actcagatcg ccgagggcaa gctggttctc cataaccaag gcttcgttca tgagcaggtc 420
 cgcaagtacg gcgccaaggt caacaagacc gtctcgccta ccaggtcat gaactactac 480
 accgaagagc aggtcccagt tctgacctcg ctggtgcaaa actacctgac cttcaaccac 540
 tggcactcgg atattcctgg caacaccaac cccaaccgtg ccgcccgtgt ttctggtacc 600
 tccctcggcc acggtctgaa cgacgctgct tttaaccaagc acgagttccc ccagcggtec 660
 atcttccagc agttgaccga gaccggtcac tcgtggatca actatgtcga cctgcccggc 720
 ggtaccggcc ctgatgcagg ctacttcaac tggacctatg ctaccaacaa ccaagacaag 780
 atccagcccc tggccaagtt ctacactgac gctgcccgtg gtgcccttcc cgaattggcg 840
 tacctcaatc cctcgtgctg cggagttggc accacctcca tgcactctgc cggcctgac 900
 tccgatggcg aggttttcat caagaaggtc tacgatgctc tgcgcgccgg tccccagtgg 960
 gaggagacct tcttcatcct gaccttcgac gagagcgggt gcttccacga ccacgtctcc 1020
 gctcctctcg ctccccggcc ggataacctg acctttactc tcagcaccac cagtggcgaa 1080
 gactacacct tcccccttga ccgtctgggt ggtcgcatcc cgaccctgct gatctcccc 1140
 tgggttgcca aggacaggt cgagcagaag ggtaagaatg ctcacggcga gactgtgtcc 1200
 tactcggctg catcgattct gggactctg ggctacctct gggactttga gccctttacc 1260
 cctcgcgtcg agcatgcggc gtcatttgag cacttgatc gtctccatgc tcggaaggac 1320
 actccactg cgctcccttc tgcagtgcct ttcaggaata gaaacgtgta g 1371

<210> 6<211> 456<212> PRT<213> Aspergillus fumigatus<400> 6

8

Met His Pro Ser Ala Leu Leu Gly Leu Leu Ala Phe Thr Val Ala Ala
 1 5 10 15
 Ser Ala Ile Pro Ala Pro Pro Ala Ser Ala His Ser Lys His Ser Ile
 20 25 30
 Asn Asn Phe Lys Ser Lys Ile Lys Asn Val Val Ile Leu Val Met Glu
 35 40 45
 Asn Arg Ser Phe Asp Asn Leu Leu Gly Gly Gln Lys Leu Lys Gly Leu
 50 55 60
 Glu Asn Pro Val Asn Thr Gly Pro Tyr Cys Asn Pro Tyr Asn Leu Thr
 65 70 75 80
 Asp Pro Ser Gln Gly Val Ala Cys Ser Ala Ala Lys Asp Phe Asp Ser
 85 90 95
 Ile Thr Asn Asp Pro Asp His Ala Ile Tyr Gly Asn Asn Ile Glu Phe
 100 105 110
 Tyr Gly Thr Phe Asn Pro Asp Asn Thr Gln Ile Ala Glu Gly Lys Leu
 115 120 125
 Val Pro His Asn Gln Gly Phe Val His Glu Gln Val Arg Lys Tyr Gly
 130 135 140
 Ala Lys Val Asn Lys Thr Val Leu Ala Thr Gln Val Met Asn Tyr Tyr
 145 150 155 160
 Thr Glu Glu Gln Val Pro Val Leu Thr Ser Leu Val Gln Asn Tyr Leu
 165 170 175
 Thr Phe Asn His Trp His Ser Asp Ile Pro Gly Asn Thr Asn Pro Asn
 180 185 190
 Arg Ala Ala Leu Val Ser Gly Thr Ser Leu Gly His Gly Leu Asn Asp
 195 200 205
 Ala Ala Phe Thr Lys His Glu Phe Pro Gln Arg Ser Ile Phe Gln Gln
 210 215 220
 Leu Thr Glu Thr Gly His Ser Trp Ile Asn Tyr Val Asp Pro Ala Gly
 225 230 235 240
 Gly Thr Gly Pro Asp Ala Gly Tyr Phe Asn Trp Thr Tyr Ala Thr Asn
 245 250 255
 Asn Gln Asp Lys Ile Gln Pro Leu Ala Lys Phe Tyr Thr Asp Ala Ala
 260 265 270
 Ala Gly Ala Leu Pro Glu Leu Ala Tyr Leu Asn Pro Ser Cys Cys Gly
 275 280 285
 Val Gly Thr Thr Ser Met His Pro Ala Gly Leu Ile Ser Asp Gly Glu
 290 295 300
 Ala Phe Ile Lys Lys Val Tyr Asp Ala Leu Arg Ala Gly Pro Gln Trp
 305 310 315 320

Glu Glu Thr Leu Phe Ile Leu Thr Phe Asp Glu Ser Gly Gly Phe His
325 330 335

Asp His Val Ser Ala Pro Leu Ala Pro Arg Pro Asp Asn Leu Thr Phe
340 345 350

Thr Leu Ser Thr Pro Ser Gly Glu Asp Tyr Thr Phe Pro Phe Asp Arg
355 360 365

Leu Gly Gly Arg Ile Pro Thr Leu Leu Ile Ser Pro Trp Val Ala Lys
370 375 380

Ala Gln Val Glu Gln Lys Gly Lys Asn Ala His Gly Glu Thr Val Ser
385 390 395 400

Tyr Ser Ala Ala Ser Ile Leu Arg Thr Leu Gly Tyr Leu Trp Asp Phe
405 410 415

Glu Pro Phe Thr Pro Arg Val Glu His Ala Ala Ser Phe Glu His Leu
420 425 430

Ile Arg Leu His Ala Arg Lys Asp Thr Pro Thr Ala Leu Pro Ser Ala
435 440 445

Val Pro Phe Arg Asn Arg Asn Val
450 455

<210> 7<211> 3062<212> DNA<213> Aspergillus fumigatus<400> 7
 ttacttaaat aaattgccag aaacttttcc tctccttacc ggtttctcta aatttaactg 60
 ctagtccacac tgggacagtt ggaattgcgg cctagttggt ggggccacgg gtttctctat 120
 gcctgagagt cgagaagggt ctgatgatag gccgctcggt catctcaata gccaaagtga 180
 tgcctagaca aaacaagaca ttcagacgag acattttaatt ccttcaacca gctcaatcct 240
 catcatgcaa gatcaacatg cttgaatgat cagatatgat tggcgagtgc agatacaata 300
 cagaaacgag taagcaaadc agatcgcgca gcaagaggag tgctctctct tacatgctct 360
 gattggctga tacaaggcac cgtcgtggag acaagccaag gaacgccatg aaagotttagc 420
 ggcccacgtg tatttattgt attcctcctt cctgttctctg atccactgca cataggtaag 480
 taataatgta atgacatcga acgtgtaaaa cactcatacc ggcggttagca atctcggggtt 540
 gaatttagca gtgctacctt gaggaatggt acagatgaat aaagcaataa gcccgctgat 600
 ctcaatgtaa aagctcctca atcagcccca cctattggga caatctacca cagatgccat 660
 caactgcaag acccgactgt attactggat gtgaggggag atatcgctta ccggtaggca 720
 tgtcagatat gacctcagt ctaacataat gctgcgaaat gcctgcctat aagatttgctc 780
 gatcagcgca tgtgctggcg aatgtcaccc ttcatttggt catccagcat atcatattga 840
 tagcaggacg ttgtggtatc agtgggggta caagactacc tgccttagta taaaagatgg 900

ccaaactgct cagcaagtga caatgggtaa gagctgcgtc catcatcatg aaggccaaca 960
ctctgggtgcc tttgctcggc ctctccagca gcgctgccgc tgcggccgtg gctgcgtccg 1020
gcaatacccc ttttgggtac gcctctggct ccaaggagtc cattgccaac ttgaaggata 1080
agattgagaa tgtcgtgtgg attctgctgg agaatcgag cttcgacaac atcctgggtg 1140
gtgtcaagcg taagggtctg gacaacgttg ttaacaacgg tcccttctgc aacccccaga 1200
atgtgagcga tcccagcagc accaagtggc gctcgtctca caaggacttt gattcagtca 1260
aacatgacct cgatcactcg gtcactgggt acaacatgga gttgtatggc acgtatactc 1320
ccagtaatga tgccatcgcc aacggcacc tgaagccac catgaacggc tttgtggagc 1380
agcagctggc gcatcacaag ggtcttgatc ccaagggtggc cgatgaggaa gtcattgggt 1440
actactccga ggacgagatc cccacgctgg tcaacctggc tgatgagttc accaccttca 1500
actactggca ctgctgtgtc cctgggtgaa gtcacgaacc ctactacgag tacaagagac 1560
tgatgagaat agcccaccaa cccaaccgg ctgtgcgccc tctcgggtac ctccgatggc 1620
cacggaacca acgacaacag cttcgatgtc tctggcgctc acatctccag catcttccag 1680
gttgccagcg agaaggacat cacctggaag aactacgagc gcaccaacgg cgccttcctt 1740
tcggacgctc tcttcttcaa ctggaccgcc caaacgccca agcataacgt cgtccctctc 1800
gagaacttct accaggacgc ctatctcgcc ctcttcccc agctctccta catcaacccc 1860
tcttctgctg acctcgacac caactccatg caccctccg gcaacgtctc cttcggccag 1920
gtcctcgtca aacagatcta cgatgctgtt cgcaccggac cccagtggga caagaccctc 1980
ctactcgtca ccttcgacga gaccggcggt ttctacgacc acgtcgagcc cctcttggc 2040
gtccgcccag atagcaagac ctacaccgag accgctgccg acggcagctc gtacacctc 2100
aactttgacc gtctggggcg ccgtatgcct acctggctga tctctccgta tacgcccag 2160
ggccacattg agaactacgg tgtcgacct gccaccggca agtcagcctc gtacagtgcc 2220
acttcogtgc tcaaaacctc tggatacctc tgggatctgg atgactttac tccccgtgtc 2280
tctcactcgc ctgcttttga ccacctgac ggtcctaaga agcgcaatac tctgccagc 2340
ttggcgaatc cccatccttt cctgatgct gtttagattt catgcaaac ctgctcagaa 2400
aaggagatgt ctcaagagga gattcaaagt attccacttt gccctttgta ctttgtatat 2460
gatataatct catcctgatg ctattgaata tggcttact ttactggtgg tgttgatgtt 2520
caatattgaa tgagcctgaa ttctggacaa caaatgattt ttacaagtga gggctgaact 2580
gatccgccac ctttttttca tttggagctc ctctccagta acttcataaa agtaaaaaa 2640
gagatgcgag tttctcctgc ttggctactc cggaacaacg caggagcgca tctgtatccc 2700

ctttgaccag cctaattgtac aagaggccag tcgcatagaa ttacctctac attttccggc 2760
 agcggagaag agtagggctt tcccactctc ttccatttag gatcctcgct ccattcacc 2820
 agtgagacct atagctccgc acagctctta cttgggggtga aggacaagga atcgtcagcc 2880
 taccttgtat atttttgctc tttcatttcc acaaactcaa cacgctccat ctccacctca 2940
 gctttccagc aacattaacc tctccacgaa cttatctgcg gctccctcca acagctccgg 3000
 ccaatgtcaa actgcgtcca gaatgtcgct tttcccatta tcagagcaac gccgaatatc 3060
 at 3062

<210> 8<211> 1383<212> DNA<213> *Aspergillus fumigatus*<400> 8
 atgaaggcca acactctggt gcctttgctc ggtctctcca gcagcgctgc cgctgcggcc 60
 gtggctgcgt cgggcaatac cccttttggg tacgcctctg gctccaagga gtccattgcc 120
 aacttgaagg ataagattga gaatgtcggt tggattctgc tggagaatcg cagcttcgac 180
 aacatcctgg gtggtgtcaa gcgtaagggt ctggacaacg ttgttaacaa cggtccttc 240
 tgcaaccccc agaattgtgag cgatcccagc agcaccaagt ggtgctcggt ctacaaggac 300
 tttgattcag tcaaacatga cccgatcac tcggtcaactg ggtacaacat ggagttgtat 360
 ggcacgtata ctcccagtaa tgatgccatc gccaacggca ccctgaagcc caccatgaac 420
 ggctttgtgg agcagcagct ggtgcatcac aagggtcttg atcccaaggt ggccgatgag 480
 gaagtcattg gctactactc cgaggacgag atccccacgc tgggtcaacct ggttgatgag 540
 ttcaccacct tcaactactg gcaactcgtgt gtccctggtc ccaccaaccc caaccggctg 600
 tgcgccctct cgggtacctc cgatggccac ggaaccaacg acaacagctt cgatgtctct 660
 ggcgtcgaca tctccagcat cttccagggt gccagcgaga aggacatcac ctggaagaac 720
 tacgacggca ccaacggcgc cttcctttcg gacgtctctt tcttcaactg gaccgcccaa 780
 aacgccaagc ataacgtcgt ccctctcgag aacttctacc aggacgcta tctcggcctc 840
 cttccccagc tctctacat caaccctct tgctgcgacc tcgacaccaa ctccatgcac 900
 ccctccggca acgtctcctt cggccaggtc ctcgtoaaac agatctaaga tgctgttcgc 960
 accggacccc agtgggacaa gaccctcta ctcgtaacct tcgacgagac cggcgggttc 1020
 tacgaccagc tcgagcccc tcttgccgtc ccccagata gcaagacct caccgagacc 1080
 gctgccgacg gcagctcgta caccttcaac tttgacogtc tgggcggcgg tatgcctacc 1140
 tggctgatct ctccgtatac gcccaagggc cacattgaga actacgggtgt cgaccctgcc 1200
 accggcaagt cagcctcgta cagtgccact tccgtgtca aaacccttgg atacctctgg 1260

12

gatctggatg actttactcc ccgtgtctct cactcgcctg cttttgacca cctgatcggt 1320
 cctaagaagc gcaatactcc tgccacgttg gcgaatcccc atcctttccc tgatgctgtt 1380
 tag 1383

<210> 9<211> 460<212> PRT<213> *Aspergillus fumigatus*<400> 9

Met Lys Ala Asn Thr Leu Val Pro Leu Leu Gly Leu Ser Ser Ser Ala
 1 5 10 15

Ala Ala Ala Ala Val Ala Ala Ser Gly Asn Thr Pro Phe Gly Tyr Ala
 20 25 30

Ser Gly Ser Lys Glu Ser Ile Ala Asn Leu Lys Asp Lys Ile Glu Asn
 35 40 45

Val Val Trp Ile Leu Leu Glu Asn Arg Ser Phe Asp Asn Ile Leu Gly
 50 55 60

Gly Val Lys Arg Lys Gly Leu Asp Asn Val Val Asn Asn Gly Pro Phe
 65 70 75 80

Cys Asn Pro Gln Asn Val Ser Asp Pro Ser Ser Thr Lys Trp Cys Ser
 85 90 95

Val Tyr Lys Asp Phe Asp Ser Val Lys His Asp Pro Asp His Ser Val
 100 105 110

Thr Gly Tyr Asn Met Glu Leu Tyr Gly Thr Tyr Thr Pro Ser Asn Asp
 115 120 125

Ala Ile Ala Asn Gly Thr Leu Lys Pro Thr Met Asn Gly Phe Val Glu
 130 135 140

Gln Gln Leu Val His His Lys Gly Leu Asp Pro Lys Val Ala Asp Glu
 145 150 155 160

Glu Val Met Gly Tyr Tyr Ser Glu Asp Glu Ile Pro Thr Leu Val Asn
 165 170 175

Leu Val Asp Glu Phe Thr Thr Phe Asn Tyr Trp His Ser Cys Val Pro
 180 185 190

Gly Pro Thr Asn Pro Asn Arg Leu Cys Ala Leu Ser Gly Thr Ser Asp
 195 200 205

Gly His Gly Thr Asn Asp Asn Ser Phe Asp Val Ser Gly Val Asp Ile
 210 215 220

Ser Ser Ile Phe Gln Val Ala Ser Glu Lys Asp Ile Thr Trp Lys Asn
 225 230 235 240

Tyr Asp Gly Thr Asn Gly Ala Phe Leu Ser Asp Ala Leu Phe Phe Asn
 245 250 255

Trp Thr Ala Gln Asn Ala Lys His Asn Val Val Pro Leu Glu Asn Phe
 260 265 270

Tyr	Gln	Asp	Ala	Tyr	Leu	Gly	Leu	Leu	Pro	Gln	Leu	Ser	Tyr	Ile	Asn	
	275								280						285	
Pro	Ser	Cys	Cys	Asp	Leu	Asp	Thr	Asn	Ser	Met	His	Pro	Ser	Gly	Asn	
	290					295					300					
Val	Ser	Phe	Gly	Gln	Val	Leu	Val	Lys	Gln	Ile	Tyr	Asp	Ala	Val	Arg	
305					310					315					320	
Thr	Gly	Pro	Gln	Trp	Asp	Lys	Thr	Leu	Leu	Leu	Val	Thr	Phe	Asp	Glu	
				325					330					335		
Thr	Gly	Gly	Phe	Tyr	Asp	His	Val	Glu	Pro	Pro	Leu	Ala	Val	Arg	Pro	
			340					345					350			
Asp	Ser	Lys	Thr	Tyr	Thr	Glu	Thr	Ala	Ala	Asp	Gly	Ser	Ser	Tyr	Thr	
		355					360					365				
Phe	Asn	Phe	Asp	Arg	Leu	Gly	Gly	Arg	Met	Pro	Thr	Trp	Leu	Ile	Ser	
	370					375					380					
Pro	Tyr	Thr	Pro	Lys	Gly	His	Ile	Glu	Asn	Tyr	Gly	Val	Asp	Pro	Ala	
385					390					395					400	
Thr	Gly	Lys	Ser	Ala	Ser	Tyr	Ser	Ala	Thr	Ser	Val	Leu	Lys	Thr	Leu	
			405						410					415		
Gly	Tyr	Leu	Trp	Asp	Leu	Asp	Asp	Phe	Thr	Pro	Arg	Val	Ser	His	Ser	
			420					425					430			
Pro	Ala	Phe	Asp	His	Leu	Ile	Gly	Pro	Lys	Lys	Arg	Asn	Thr	Pro	Ala	
	435						440					445				
Thr	Leu	Ala	Asn	Pro	His	Pro	Phe	Pro	Asp	Ala	Val					
	450					455					460					

<210>	10<211>	315<212>	DNA<213>	Aspergillus nidulans<400>	10	
atgataacga	ccattctatc	atatactcta	gcgttctcag	ttcggttaagc	catatatcca	60
tcattctcatc	tcgttataatc	caaaagacaa	tacaagaaag	aaagccaata	aatacaatga	120
aggttgcgga	accagctccg	ttagaaaata	acaggctccg	gcagaagttc	aggcgtatcc	180
tctctaaact	tcttctcaat	cagatctcca	aaactggcag	accactcaac	tctcggcgta	240
aggatatcca	atccccacaa	ctcatccaca	tacttcaaga	tcgacgtatg	cgtgtactcc	300
cccttcgctt	gccct					315

<210>	11<211>	175<212>	DNA<213>	Aspergillus nidulans<400>	11	
agggcaagcg	aagggggagt	acacgcatac	gtcgatcttg	aagtatgtgg	atgagttgtg	60
gggattggat	atccttacgc	cgagagttga	gtggtctgcc	agttttggag	atctgattga	120
gaagaagttt	agagaggata	cgootgaact	tctgccggag	cctgttattt	tctaa	175

14

<210> 12<211> 57<212> PRT<213> Aspergillus nidulans<400> 12

Gly Gln Ala Lys Gly Glu Tyr Thr His Thr Ser Ile Leu Lys Tyr Val
 1 5 10 15

Asp Glu Leu Trp Gly Leu Asp Ile Leu Thr Pro Arg Val Glu Trp Ser
 20 25 30

Ala Ser Phe Gly Asp Leu Ile Glu Lys Lys Phe Arg Glu Asp Thr Pro
 35 40 45

Glu Leu Leu Pro Glu Pro Val Ile Phe
 50 55

<210> 13<211> 561<212> DNA<213> Aspergillus niger<220><221>
 misc_feature<223> n is A, G, C or T

<400> 13
 cctcgccgcga gtggaacgag actctcttca tcttgacctt tgatgagcac ggcggttttg 60
 cagaccacgt tccccctccc gagaatgtgc cccaggtga ccatgcact cataaagagt 120
 gggctctgga tggaagcaag attgagttca agttcgaccg gctgggaatc cgcgtcccta 180
 cgctgctgat gtctccctgg gttggcaagg gtgtcgtaca aaaccgtccc acaaatacagc 240
 caaacgattt caccataacc tcgatcttga agtatctttc agaactgtgg gacttgcccg 300
 tgtaaacgcc gagagtcgcg tggtcgcca gtttcggggg cttgatcacc actactatc 360
 gggatgatac ccccgataaa ctctctgagc ctgttgactt ttagatggca tacgagggat 420
 gagatattat gaatgtaatg actanantga nccatatntt acatgctttt ttaatatgat 480
 atnggctgag gactggatnn ngctcgggtc atatgaaata ctgnatnagt natctattat 540
 cgtncatagt nataantatg g 561

<210> 14<211> 133<212> PRT<213> Aspergillus niger<400> 14

Ser Pro Gln Trp Asn Glu Thr Leu Phe Ile Leu Thr Phe Asp Glu His
 1 5 10 15

Gly Gly Phe Ala Asp His Val Pro Pro Pro Glu Asn Val Pro Pro Gly
 20 25 30

Asp His Arg Thr His Lys Glu Trp Ala Leu Asp Gly Ser Lys Ile Glu
 35 40 45

Phe Lys Phe Asp Arg Leu Gly Ile Arg Val Pro Thr Leu Leu Met Ser
 50 55 60

Pro Trp Val Gly Lys Gly Val Val Gln Asn Arg Pro Thr Asn Gln Pro
 65 70 75 80

Asn Asp Phe Thr His Thr Ser Ile Leu Lys Tyr Leu Ser Glu Leu Trp
 85 90 95

15

Asp Leu Pro Val Leu Thr Pro Arg Val Ala Trp Ser Pro Ser Phe Gly
 100 105 110

Gly Leu Ile Thr Thr Thr Tyr Arg Asp Asp Thr Pro Asp Lys Leu Pro
 115 120 125

Glu Pro Val Asp Phe
 130

<210> 15<211> 517<212> DNA<213> Magnaporthe grisea<400> 15
 gcttttgtat tgtatcaatc atgaaggcta atactttgat tccccttctg gggtttatcag 60
 gcaactgctct ggggtgctgct gtcacccgtg gcaatcaaga gccttggggc tataagtccg 120
 gttccaagga atccattgcc aatctaaagg acaaagtcga gaatgtcgtc tggattctgt 180
 tggaaaatcg cggcttcgac aacatgctgg gtgggtgtcca caaagagggc ctcgacaatg 240
 tcgtcaacaa cgggccattc tgcaatcctg agtctgtcaa ccaaaccac agcaagaagt 300
 ggtgcagtac tttcaaggat tatgactccg tcattcaoga cctgatcat tcagtcactg 360
 gtgtcaactt tgagatgtac ggcacctata acccatctaa caaggatatt gcgagcggca 420
 aattgaagcc taatctaaac ggctttgtcg agcgccagat ggattcctac ccagctatct 480
 cggctaagcg tgccactgaa gaggttatgg cctacta 517

<210> 16<211> 165<212> PRT<213> Magnaporthe grisea<400> 16

Met Lys Ala Asn Thr Leu Ile Pro Leu Leu Gly Leu Ser Gly Thr Ala
 1 5 10 15

Leu Gly Ala Ala Val Thr Arg Gly Asn Gln Glu Pro Trp Gly Tyr Lys
 20 25 30

Ser Gly Ser Lys Glu Ser Ile Ala Asn Leu Lys Asp Lys Val Glu Asn
 35 40 45

Val Val Trp Ile Leu Leu Glu Asn Arg Gly Phe Asp Asn Met Leu Gly
 50 55 60

Gly Val His Lys Glu Gly Leu Asp Asn Val Val Asn Asn Gly Pro Phe
 65 70 75 80

Cys Asn Pro Glu Ser Val Asn Gln Thr His Ser Lys Lys Trp Cys Ser
 85 90 95

Thr Phe Lys Asp Tyr Asp Ser Val Ile His Asp Pro Asp His Ser Val
 100 105 110

Thr Gly Val Asn Phe Glu Met Tyr Gly Thr Tyr Asn Pro Ser Asn Lys
 115 120 125

Asp Ile Ala Ser Gly Lys Leu Lys Pro Asn Leu Asn Gly Phe Val Glu
 130 135 140

Arg Gln Met Asp Ser Tyr Pro Ala Ile Ser Ala Lys Arg Ala Thr Glu

16

145	150	155	160
Glu Val Met Ala Tyr			
165			
<210> 17<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	17
ccattatgcc tggagtcggt			20
<210> 18<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	18
agcctgagcc gtgaatagaa			20
<210> 19<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	19
accatgactg tcctgtcgct			20
<210> 20<211> 18<212>	DNA<213>	Aspergillus fumigatus<400>	20
atccgctggc tcaggtaa			18
<210> 21<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	21
cagtattgac gggcttgtga			20
<210> 22<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	22
ccggtcgtgt tggagtagtt			20
<210> 23<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	23
cagactgtcc agtccgatca			20
<210> 24<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	24
aagtgccgga cgtcagatag			20
<210> 25<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	25
atcatcgacc tgctgattcc			20
<210> 26<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	26
ttttttcatc ttccggtgt			20
<210> 27<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	27
tgcagaatgc atcctagtgc			20
<210> 28<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	28
cctgaaaggc actgcagaag			20
<210> 29<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	29
gacottcaac cactggcact			20
<210> 30<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	30
gaagagggtc tcctcccact			20

<210> 31<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	31	
cccttactgc aaccctaca				20
<210> 32<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	32	
gggaactcgt gcttggtaaa				20
<210> 33<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	33	
caacaacaac aacggtcagc				20
<210> 34<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	34	
atgatattcg gcgttgctct				20
<210> 35<211> 21<212>	DNA<213>	Aspergillus fumigatus<400>	35	
atgaaggcca aactctggt g				21
<210> 36<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	36	
acagcatcag ggaaaggatg				20
<210> 37<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	37	
tcaactcggtc actgggtaca				20
<210> 38<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	38	
gcggtccagt tgaagaagag				20
<210> 39<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	39	
gtcaagcgta agggctctgga				20
<210> 40<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	40	
aagctgttgt cgttgggtcc				20
<210> 41<211> 26<212>	DNA<213>	Aspergillus fumigatus<400>	41	
cgccgtgggc tgtgcgcaca ccattg				26
<210> 42<211> 22<212>	DNA<213>	Aspergillus fumigatus<400>	42	
ctgggggagc gccgacctgt cg				22
<210> 43<211> 25<212>	DNA<213>	Aspergillus fumigatus<400>	43	
gcggggacga gcggagcgcc tcgta				25
<210> 44<211> 25<212>	DNA<213>	Aspergillus fumigatus<400>	44	
acttcgacag gtcggcgctc cccca				25
<210> 45<211> 26<212>	DNA<213>	Aspergillus fumigatus<400>	45	

cgaggcgctc cgctcgtccc cgcagt		26
<210> 46<211> 27<212> DNA<213> Aspergillus fumigatus<400> 46 cgcggaaccac gtgccgcccc cggagaa		27
<210> 47<211> 22<212> DNA<213> Aspergillus fumigatus<400> 47 ggttgcagta agggccggtg tt		22
<210> 48<211> 23<212> DNA<213> Aspergillus fumigatus<400> 48 gccctcggcg atctgagtgt tgt		23
<210> 49<211> 22<212> DNA<213> Aspergillus fumigatus<400> 49 tccagcccct ggccaagttc ta		22
<210> 50<211> 22<212> DNA<213> Aspergillus fumigatus<400> 50 gaatgctcac ggcgagactg tg		22
<210> 51<211> 22<212> DNA<213> Aspergillus fumigatus<400> 51 gtacggcgcc aaggtcaaca ag		22
<210> 52<211> 25<212> DNA<213> Aspergillus fumigatus<400> 52 ccatgacttc ctcatcggcc acctt		25
<210> 53<211> 25<212> DNA<213> Aspergillus fumigatus<400> 53 gttggtgtcg aggtcgcagc aagag		25
<210> 54<211> 25<212> DNA<213> Aspergillus fumigatus<400> 54 cgttggtgcc gtcgtagttc ttcca		25
<210> 55<211> 25<212> DNA<213> Aspergillus fumigatus<400> 55 aggtcgcagc aagaggggtt gatgt		25
<210> 56<211> 25<212> DNA<213> Aspergillus fumigatus<400> 56 aaggccaaca ctctggtgcc tttgc		25
<210> 57<211> 25<212> DNA<213> Aspergillus fumigatus<400> 57 ccaaggtggc cgatgaggaa gtcac		25
<210> 58<211> 25<212> DNA<213> Aspergillus fumigatus<400> 58 cttgctgoga cctcgacacc aactc		25
<210> 59<211> 25<212> DNA<213> Aspergillus fumigatus<400> 59 atacgcccaa gggccacatt gagaa		25

19

<210> 60<211> 19<212>	DNA<213>	Aspergillus fumigatus<400>	60	19
gaatgtcgtg gttctggtg				
<210> 61<211> 17<212>	DNA<213>	Aspergillus fumigatus<400>	61	17
gcgacatcag caccgtc				
<210> 62<211> 22<212>	DNA<213>	Aspergillus fumigatus<400>	62	22
ccagcagatg ttcgaccca ag				
<210> 63<211> 24<212>	DNA<213>	Aspergillus fumigatus<400>	63	24
cagtgaactc catctcgtcc atac				
<210> 64<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	64	20
cggcgaggtc atcaactact				
<210> 65<211> 19<212>	DNA<213>	Aspergillus fumigatus<400>	65	19
agctgctcga agatggaac				
<210> 66<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	66	20
ttcgactcca tcaccaatga				
<210> 67<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	67	20
ccagtgggtg aaggtcaggt				
<210> 68<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	68	20
gatctacgat gctgttcgca				
<210> 69<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	69	20
agagatcagc caggtaggca				
<210> 70<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	70	20
ggcagaagtt caggcgtatc				
<210> 71<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	71	20
gaagggggag tacacgcata				
<210> 72<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	72	20
atcagccaaa cgatttcacc				
<210> 73<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	73	20
ggggatatcat cccgataggt				
<210> 74<211> 20<212>	DNA<213>	Aspergillus fumigatus<400>	74	20
gacaatgtcg tcaacaacgg				

20

<210> 75<211> 20<212> DNA<213> Aspergillus fumigatus<400> 75 tgaatgatca gggtcgtgaa	20
<210> 76<211> 23<212> DNA<213> Aspergillus fumigatus<400> 76 caccatgcct catgtgcccc acg	23
<210> 77<211> 22<212> DNA<213> Aspergillus fumigatus<400> 77 gaaatccgct ggctcaggta ac	22
<210> 78<211> 23<212> DNA<213> Aspergillus fumigatus<400> 78 caccatgatt cctgcaccgc cag	23
<210> 79<211> 22<212> DNA<213> Aspergillus fumigatus<400> 79 cacgtttcta ttcctgaaag gc	22
<210> 80<211> 24<212> DNA<213> Aspergillus fumigatus<400> 80 caccatggct gcgtccggca atac	24
<210> 81<211> 22<212> DNA<213> Aspergillus fumigatus<400> 81 aacagcatca gggaaaggat gg	22
<210> 82<211> 5387<212> DNA<213> Aspergillus fumigatus<400> 82 tcgcgcggtt cggatgatgac ggtgaaaacc tctgacacat gcagctcccg gagacgggtca	60
cagcttgtct gtaagcggat gccgggagca gacaagcccg tcagggcgcg tcagcgggtg	120
ttggcggtg tcggggctgg cttaactatg cggcatcaga gcagattgta ctgagagtgc	180
accatatgcg gtgtgaaata ccgcacagat gcgtaaggag aaaataccgc atcaggcgcc	240
attogccatt caggctgcgc aactgttggg aagggcgatc ggtgcgggcc tcttcgctat	300
tacgacagct gtctcttata cacatctcaa ccatcatcga tgaattttct cgggtgttct	360
cgcatattgg ctgcaattcg agctcggtag ccggggatcc tctagaagtc ctgaatagta	420
gtttgtggat taacattgtt ccgatgtagg aatcatgatc ccaaccagaa gagctggaca	480
gcccctcttc cagagcattt ttggtgggat gttttggctt agtgcgatgc aactggacaa	540
agtccttccg tttctactgc gtcttacatc atctggtatc tacgcaagcc gccacttac	600
catatgaata agaggcactc aggttttccc tcaccccccc gaagcgatgg taagcgggtg	660
ccaaatgcat cgggagtttc tctatcataa taacctaggt attccgtaat ctattaccag	720
tctttccgaa gagctggtag caactgcacg agatttgtag gagcgagtac ccggctggac	780
gagcacgcag cacggctatt ggtcagcatg gtagctaccg aggggaggca ggccgccccaa	840
atatcgtgag tctcctgctt tgcccgggtg atgaaaccgg aaaagctgct atagagcttc	900

tgggcgggcgc atgtcgggaa accagcagca agctgaccca gaaagacccg tcctcaagcc	960
attaccgtac taatcaatta tttgtgtagc aacactggga agctgtagtg cataggctgg	1020
agcagctatt tggccttttag ccccgctctgt ccgcccgggtg tgcggtttcg actggcgcg	1080
aagctcaagg tgatcaggtc gttgcgtcag tcggagacaa caagccattg cctttttctac	1140
tgccccctccc ccgctgggtg cctttttctc tcattctctc ctctcttccc atcatcagca	1200
tcattaatct actgtctctc tttctttcta tcattctata aagtaagaac atatccatct	1260
tcctcaatc ccgtctacaa tagtgtctc ttcactactc tgtctctatc totcaaagct	1320
tgactgacat ttaccccgct cagtaccaga cgaatctaca cagaattcga gctcactaaa	1380
ccatggccaa gttgaccagt gccgttcggtg tgctcaccgc gcgcgacgtc gccggagcgg	1440
tcgagttctg gaccgaccgg ctccgggttct cccgggaactt cgtggaggac gacttcgccg	1500
gtgtgggtccg ggacgacgtg accctgttca tcagcgcggt ccaggaccag gtgggtgccg	1560
acaacaccct ggctgggtg tgggtgcgcg gcctggacga gctgtacgcc gagtgggtcg	1620
aggtcgtgtc cacgaaactc cgggacgcct ccgggcccgc catgaccgag atcggcgagc	1680
agccgtgggg gcgggagttc gccctgcgcg acccggcgg caactgcgtg cacttcgtgg	1740
ccgaggagca ggactgagaa ttccactagt gcagaaagct gttttccttg ctctgtggta	1800
taagtctagt gccactattc tatgatgagt tgatgactct ttcattgactg gaaggcttac	1860
attctccaag atcatgtctc actcaaaaact tatctcgggt tcactttcgg gttccatata	1920
tctcatcatt tctgggttta gaaacatctc tctcgttttt gcagctcttc tacgtactcc	1980
tagcggtttc actgaaatga atacatttgg gtaacctaat tgccaattca tatcttcctg	2040
agggcagtaa cacatcacgt acattctatc agctgtgata gagttacaaa actagcaata	2100
cttttatgct tcctcctttc ttaccattta cacatccgct ttctctctgc tcttgatctt	2160
ggccctgat tgtattgtca cctcaccaa ttcaagtcac cacctcttct ctagagtoga	2220
cttttatgga cagcaagcga accggaattg ccagctgggg cgcctctgga taagggtggg	2280
aagccctgca aagtaaaactg gatggctttc tcgccgcaa ggatctgatg gcgcagggga	2340
tcaagctctg atcaagagac aggatgagga tcgtttcgca tgattgaaca agatggattg	2400
cacgcaggtt ctccggccgc ttgggtggag aggctattcg gctatgactg ggcacaacag	2460
acaatoggct gctctgatgc ccgctgttc cggctgtcag cgcagggggc cccggttctt	2520
tttgtcaaga ccgacctgtc cgggtgccctg aatgaactgc aagacgaggc agcgcggcta	2580
tcgtgggtgg ccacgacggg cgttccttgc gcagctgtgc tcgacgttgt cactgaagcg	2640

ggaagggact ggctgctatt gggcgaagtg ccggggcagg atctcctgtc atctcacctt 2700
gctcctgccg agaaagtatc catcatggct gatgcaatgc ggcggtgca tacgcttgat 2760
ccggctacct gccattcga ccaccaagcg aaacatcgca tcgagcgagc acgtactcgg 2820
atggaagccg gtcttgtcga tcaggatgat ctggacgaag agcatcaggg gctcgcgcc 2880
gccgaactgt tcgccaggct caaggcgagc atgcccgacg gcgaggatct cgtcgtgacc 2940
catggcgatg cctgcttgcc gaatatcatg gtggaaaatg gccgcttttc tggattcatc 3000
gactgtggcc ggctgggtgt ggcgaccgc tatcaggaca tagcgttggc taccogtgat 3060
attgctgaag agcttggcgg cgaatgggct gaccgcttcc tcgtgcttta cggtatcgcc 3120
gctcccgatt cgcagcgcat cgccttctat cgccttcttg acgagttctt ctgaattatt 3180
aacgcttaca atttcctgat gcggtatttt ctgcgatgca tcaactagtga attcgcggcc 3240
gcctgcaggt cgacctgcag gcatgcaagc ttgccaaoga ctacgcacta gccacaaga 3300
gcttcaggt tgagatgtgt ataagagaca gctgtcttaa tgaatcggcc aacgcgcggg 3360
gagaggcggc ttgcgtattg ggcgctcttc cgttctctcg ctcaactgact cgtcgcgctc 3420
ggtcgttcgg ctgcggcgag cggtatcagc tcaactcaaag gcggtaatac ggttatccac 3480
agaatcaggg gataacgcag gaaagaacat gtgagcaaaa ggccagcaaa aggccaggaa 3540
ccgtaaaaag gccgcgttgc tggcgttttt ccataggctc cgccccctg acgagcatca 3600
caaaaatcga cgctcaagtc agaggtggcg aaacccgaca ggactataaa gataccaggc 3660
gtttccccct ggaagctccc tcgtgcgctc tctgttccg accctgccgc ttaccggata 3720
cctgtccgcc tttctccctt cgggaagcgt ggcgctttct catagctcac gctgtaggta 3780
tctcagttcg gtgtaggtcg ttcgctccaa gctgggctgt gtgcacgaac cccccgttca 3840
gccgaaccgc tgcgccttat ccggttaacta tcgtcttgag tccaaccgg taagacacga 3900
cttatcgcca ctggcagcag ccaactggtaa caggattagc agagcgaggt atgtaggcgg 3960
tgctacagag ttcttgaagt ggtggcctaa ctacggctac actagaagga cagtatttgg 4020
tatctgcgct ctgctgaagc cagttacctt cggaaaaaga gttggtagct cttgatccgg 4080
caaacaaacc accgctggta gcggtggttt ttttgtttgc aagcagcaga ttacgcgcag 4140
aaaaaaagga tctcaagaag atcctttgat cttttctacg gggctctgacg ctcaagtggaa 4200
cgaaaactca cgttaaggga ttttggtcat gagattatca aaaaggatct tcacctagat 4260
ccttttaaata taaaaatgaa gttttaaatc aatctaaagt atatatgagt aaacttggtc 4320
tgacagttac caatgcttaa tcagtgggc acctatctca gcgatctgtc tatttcgttc 4380
atccatagtt gcctgactcc ccgtcgtgta gataactacg atacgggagg gcttaccatc 4440

tggccccagt gctgcaatga taccgcgaga cccacgctca ccggtccag atttatcagc 4500
aataaaccag ccagccggaa gggccgagcg cagaagtggc cctgcaactt tatccgcctc 4560
catccagtct attaattggt gccgggaagc tagagtaagt agttcgccag ttaatagttt 4620
gcgcaacggt gttgccattg ctacaggcat cgtggtgtca cgctcgctgt ttggtatggc 4680
ttcattcagc tccggttccc aacgatcaag gcgagttaca tgatcccca tggtgtgcaa 4740
aaaagcggtt agtccttcg gtccctccgat cgttgtcaga agtaagttgg ccgcagtgtt 4800
atcactcatg gttatggcag cactgcataa ttctcttact gtcattgcat ccgtaagatg 4860
cttttctgtg actggtgagt actcaaccaa gtcattctga gaatagtga tgcggcgacc 4920
gagttgctct tgcccggcgt caatacggga taataccgcg ccacatagca gaactttaaa 4980
agtgtcatc attgaaaaac gttcttcggg gcgaaaactc tcaaggatct taccgctgtt 5040
gagatccagt tcgatgtaac cactcgtgc acccaactga tcttcagcat cttttacttt 5100
caccagcggt tctgggtgag caaaaacagg aaggcaaaat gcgcacaaaa agggaataag 5160
ggcgacacgg aaatgttgaa tactcatact cttccttttt caatattatt gaagcattta 5220
tcagggttat tgtctcatga gcggatacat atttgaatgt atttagaaaa ataaacaaat 5280
aggggttccg cgcacatttc cccgaaaagt gccacctgac gtctaagaaa ccattattat 5340
catgacatta acctataaaa ataggcgat cagaggccc tttcgtc 5387

SEQ ID No. 83: GGTATTGAGGGTCGC GCG CCT CAT GTG CCC CAC GGC AGA CTG TCC

SEQ ID No. 84: AGAGGAGAGTTAGAGCC GAA ATC CGC TGG CTC AGG TAA CG