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(54) Title: BOT1: TARGET FOR ANTIFUNGAL AGENTS

(57) Abstract: We have discovered a target protein which is conserved in fungi and essential for cell viability, cell growth, the control of cell morphogenesis, and combinations thereof. An antifungal agent targets the protein in a fungus, but not an infected host. This protein is encoded by the *bot1* gene in *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* and *Candida albicans* but is not detected in a filamentous fungus.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/19251

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : G07K 14/39
US CL : 530/371

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 530/371

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	XIANG et al. DNA sequencing and analysis of a 67.4 kb region from the right arm of	1-3
---	Schizosaccharomyces pombe chromosome II reveals 28 open reading frames including the	-----
Y	genes his5, pol5, ppa2, rip1, rpb8 and skb1. 1 July 1999 (01.07.1999), Yeast, Volume 15,	5 and 19
	pages 893-901. See entire document, especially the abstract and Table 1.	
X	EMBL: AL022305. 1 August 1998 (01.08.1998). See gene name spbc14c8.16c.	1-3
---		-----
Y		5 and 19
Y	TSAI et al. Using a eukaryotic GST fusion vector for proteins difficult to express in E.	5 and 19
	coli. 01 November 1997 (01.11.1997), BioTechniques, Volume 23, pages 794 ,796 , 798	
	and 800. See entire document.	
Y	ROMANOS, et al. Production of phosphorylated GST::HPV-6 E7 fusion protein using a	5 and 19
	yeast expression vector and glutathione S-transferase fusions. 11 January 1995	
	(11.01.1995), Gene, Volume 152, pages 137-138. See emntire document.	

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

10 October 2002 (10.10.2002)

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INTERNATIONAL SEARCH REPORT

International application No.

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Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-3, 5 and 19 with respect to SEQ ID NO:2

Remark on Protest

☐
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

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BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

The inventions listed as Groups I-XXXXIII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

To reiterate, a search of claim 1 revealed prior art that undermines the special technical feature of the invention, the polypeptide sequence of a fungal Bot1 protein. The EMBL entry (AL022305), enclosed within, identifies a sequence with 100% homology to SEQ ID No.2 of the instant application. Since claim 1 cannot overcome the prior art, unity of invention is broken in the absence of a linking special technical feature. In addition, the Markush group of SEQ ID No. 2, 4 and 6 do not possess a common chemical structure over a large portion of their structures, as indicated in Figure 1 of the instant application, thus they are not in proper Markush format. As a result, each polypeptide sequence will be considered as an independent invention. Furthermore, methods of using and methods of producing the polypeptide will be considered independent inventions in the absence of a linking special technical feature. On this basis, the inventive as set forth above correspond to different inventions for the following reasons:

The special technical feature of Group I is the isolated Bot1 polypeptide derived from *S. pombe*, identified by SEQ ID No.2, corresponding fragments and fusion polypeptides comprised of portions of that sequence, and cellular extracts comprised of said polypeptides and fragments.

The special technical feature of Group II is the polypeptide of SEQ ID No.4, representing an isolated Bot1 polypeptide derived from *S. cerevisiae*. This feature defines an advance over Group I in that the Bot1 polypeptide defined by SEQ ID No.4 is derived from a different organism and has a significantly different sequence from that of SEQ ID No.2. Because applicant is claiming the sequence based on sequence similarity alone, it is unclear that these two polypeptides are functionally related.

The special technical feature of Group III is the polypeptide of SEQ ID No.6, representing an isolated Bot1 polypeptide derived from *C. albicans*. This feature defines an advance over Group I in that the Bot1 polypeptide defined by SEQ ID No.6 is derived from a different organism and has a significantly different sequence from that of SEQ ID No.2. Because applicant is claiming the sequence based on sequence similarity alone, it is unclear that these two polypeptides are functionally related.

The special technical feature of Group IV is an artificial substrate. This feature defines an advance over Group I in that it mobilizes the polypeptide for use in analytical studies, such as the detection of molecular binding partners.

The special technical feature of Group V is an artificial substrate. This feature defines an advance over Group I in that it mobilizes the polypeptide for use in analytical studies, such as the detection of molecular binding partners.

The special technical feature of Group VI is an artificial substrate. This feature defines an advance over Group I in that it mobilizes the polypeptide for use in analytical studies, such as the detection of molecular binding partners.

The special technical feature of Group VII is the protein complex which comprises Tea1 and the polypeptide defined by SEQ ID No.2. This feature defines an advance over Group I in that it contains another polypeptide, which could affect some function of the polypeptide defined by SEQ ID No.2 as it exists in isolation, or as it exists in a complex with another polypeptide.

The special technical feature of Group VIII is the protein complex which comprises Orb6 and the polypeptide defined by SEQ ID No.2. This feature defines an advance over Group I in that it contains another polypeptide, which could affect some function of the polypeptide defined by SEQ ID No.2 as it exists in isolation, or as it exists in a complex with another polypeptide.

The special technical feature of Group IX is the protein complex which comprises both Tea1, Orb6 and the polypeptide defined by SEQ ID No.2. This feature defines an advance over Group I in that it contains at least two other polypeptides, which could affect some function of the polypeptide defined by SEQ ID No.2 as it exists in isolation or in complex with only one of the mentioned polypeptides.

The special technical feature of Group X is the protein complex which comprises a polypeptide, other than Tea1 or Orb6, and the polypeptide defined by SEQ ID No.2. This feature defines an advance over Group I in that it contains an unknown polypeptide, which could affect some function of the polypeptide defined by SEQ ID No.2 as it exists in isolation or in complex with only one of the mentioned polypeptides.

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The special technical feature of Group XI is the protein complex which comprises Tea1 and the polypeptide defined by SEQ ID No.4. This feature defines an advance over Group I in that it contains another polypeptide, which could affect some function of the polypeptide defined by SEQ ID No.4 as it exists in isolation, or as it exists in a complex with another polypeptide.

The special technical feature of Group XII is the protein complex which comprises Orb6 and the polypeptide defined by SEQ ID No.4. This feature defines an advance over Group I in that it contains another polypeptide, which could affect some function of the polypeptide defined by SEQ ID No.4 as it exists in isolation, or as it exists in a complex with another polypeptide.

The special technical feature of Group XIII is the protein complex which comprises both Tea1, Orb6 and the polypeptide defined by SEQ ID No.4. This feature defines an advance over Group I in that it contains at least two other polypeptides, which could affect some function of the polypeptide defined by SEQ ID No.4 as it exists in isolation or in complex with only one of the mentioned polypeptides.

The special technical feature of Group XIV is the protein complex which comprises a polypeptide, other than Tea1 or Orb6, and the polypeptide defined by SEQ ID No.4. This feature defines an advance over Group I in that it contains an unknown polypeptide, which could affect some function of the polypeptide defined by SEQ ID No.4 as it exists in isolation or in complex with only one of the mentioned polypeptides.

The special technical feature of Group XV is the protein complex which comprises Tea1 and the polypeptide defined by SEQ ID No.6. This feature defines an advance over Group I in that it contains another polypeptide, which could affect some function of the polypeptide defined by SEQ ID No.6 as it exists in isolation, or as it exists in a complex with another polypeptide.

The special technical feature of Group XVI is the protein complex which comprises Orb6 and the polypeptide defined by SEQ ID No.6. This feature defines an advance over Group I in that it contains another polypeptide, which could affect some function of the polypeptide defined by SEQ ID No.6 as it exists in isolation, or as it exists in a complex with another polypeptide.

The special technical feature of Group XVII is the protein complex which comprises both Tea1, Orb6 and the polypeptide defined by SEQ ID No.6. This feature defines an advance over Group I in that it contains at least two other polypeptides, which could affect some function of the polypeptide defined by SEQ ID No.6 as it exists in isolation or in complex with only one of the mentioned polypeptides.

The special technical feature of Group XVIII is the protein complex which comprises a polypeptide, other than Tea1 or Orb6, and the polypeptide defined by SEQ ID No.6. This feature defines an advance over Group I in that it contains an unknown polypeptide, which could affect some function of the polypeptide defined by SEQ ID No.6 as it exists in isolation or in complex with only one of the mentioned polypeptides.

The special technical feature of Group XIX is a molecule (which could be something other than a polypeptide) that binds to the polypeptide defined by SEQ ID No.2. This feature defines an advance over Group I in that the molecule comprises at least another molecule which could affect the activity of the polypeptide defined by SEQ ID No.2 with respect to how it functions in isolation.

The special technical feature of Group XX is a molecule (which could be something other than a polypeptide) that binds to the polypeptide defined by SEQ ID No.4. This feature defines an advance over Group I in that the molecule comprises at least another molecule which could affect the activity of the polypeptide defined by SEQ ID No.4 with respect to how it functions in isolation.

The special technical feature of Group XXI is a molecule (which could be something other than a polypeptide) that binds to the polypeptide defined by SEQ ID No.6. This feature defines an advance over Group I in that the molecule comprises at least another molecule which could affect the activity of the polypeptide defined by SEQ ID No.6 with respect to how it functions in isolation.

The special technical feature of Group XXII is the isolated *BOT1* polynucleotide derived from *S. pombe*, identified by SEQ ID No.1, corresponding fragments and chimeric polynucleotides comprised of portions of that sequence, and host cells transfected with the polynucleotide. This feature defines an advance over Group I in that a polynucleotide is a distinct molecule with a different structure and function from the polypeptide claimed in Group I.

The special technical feature of Group XXIII is the polynucleotide of SEQ ID No.3, representing an isolated *BOT1* polynucleotide derived from *S. cerevisiae*. This feature defines an advance over Group I in that a polynucleotide is a distinct molecule with a different structure and function from the polypeptide claimed in Group I.

The special technical feature of Group XXIV is the polypeptide of SEQ ID No.5, representing an isolated *BOT1* polynucleotide derived from *C. albicans*. This feature defines an advance over Group I in that a polynucleotide is a distinct molecule with a different structure and function from the polypeptide claimed in Group I.

The special technical feature of Group XXV is an artificial substrate. This feature defines an advance over Group I in that it mobilizes a polynucleotide for use in analytical studies, such as the detection of molecular binding partners, or the detection of potential orthologue based on nucleic acid hybridization.

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The special technical feature of Group XXVI is an artificial substrate. This feature defines an advance over Group I in that it mobilizes a polynucleotide for use in analytical studies, such as the detection of molecular binding partners, or the detection of potential orthologue based on nucleic acid hybridization.

The special technical feature of Group XXVII is an artificial substrate. This feature defines an advance over Group I in that it mobilizes a polynucleotide for use in analytical studies, such as the detection of molecular binding partners, or the detection of potential orthologue based on nucleic acid hybridization.

The special technical feature of Group XXVIII is an *S. pombe* cell with a mutated *BOT1* locus. This feature defines an advance over Group I because the Bot1 polypeptide produced by the cell would not be the same polypeptide as claimed in Group I, owing to mutation of the locus.

The special technical feature of Group XXIX is an *S. cerevisiae* cell with a mutated *BOT1* locus. This feature defines an advance over Group I because the Bot1 polypeptide produced by the cell would not be the same polypeptide as claimed in Group I, owing to mutation of the locus.

The special technical feature of Group XXX is a *C. albicans* cell with a mutated *BOT1* locus. This feature defines an advance over Group I because the Bot1 polypeptide produced by the cell would not be the same polypeptide as claimed in Group I, owing to mutation of the locus.

The special technical feature of Group XXXI is a method for producing a Bot1 polypeptide derived from *S. pombe*. This feature defines an advance over the art because it is a method of producing the polypeptide claimed in Group I. Because prior art was found on the isolated polypeptide claimed in Group I, applicant is not entitled to the first method of making the invention due to a lack of unity of the special technical feature.

The special technical feature of Group XXXII is a method for producing a Bot1 polypeptide derived from *S. cerevisiae*. This feature defines an advance over the art because it is a method of producing a polypeptide that is substantially different in sequence from the isolated polypeptide claimed in Group I.

The special technical feature of Group XXXIII is a method for producing a Bot1 polypeptide derived from *C. albicans*. This feature defines an advance over the art because it is a method of producing a polypeptide that is substantially different in sequence from the isolated polypeptide claimed in Group I.

The special technical feature of Group XXXIV is a process for identification of specific binding to Bot1 polypeptide derived from *S. pombe*. This feature defines an advance over Group I because it is a use of the invention claimed in Group I. Since prior art was found on the isolated polypeptide claimed in Group I, applicant is not entitled to the first method of using the invention because of a lack of unity in the special technical feature.

The special technical feature of Group XXXV is a process for identification of specific binding to Bot1 polypeptide derived from *S. cerevisiae*. This feature defines an advance over the art because it is a method of using a polypeptide that is substantially different in sequence from the isolated polypeptide claimed in Group I.

The special technical feature of Group XXXVI is a process for identification of specific binding to Bot1 polypeptide derived from *C. albicans*. This feature defines an advance over the art because it is a method of using a polypeptide that is substantially different in sequence from the isolated polypeptide claimed in Group I.

The special technical feature of Group XXXVII is a process for hybridization to *BOT1* nucleic acids derived from *S. pombe*. This feature defines an advance over Group I in that it uses a molecule that is substantially different in structure and function with respect to the molecule claimed in Group I.

The special technical feature of Group XXXVIII is a process for hybridization to *BOT1* nucleic acids derived from *S. cerevisiae*. This feature defines an advance over Group I in that it uses a molecule that is substantially different in structure and function with respect to the molecule claimed in Group I.

The special technical feature of Group XXXIX is a process for hybridization to *BOT1* nucleic acids derived from *C. albicans*. This feature defines an advance over Group I in that it uses a molecule that is substantially different in structure and function with respect to the molecule claimed in Group I.

The special technical feature of Group XXXX is a process for screening for and identifying an antifungal agent in *S. pombe*. This feature defines an advance over Group I in that it is a method of use of the polypeptide as claimed in Group I. Since prior art was found on the isolated polypeptide claimed in Group I, applicant is not entitled to a method of using the invention due to a lack of unity in the special technical feature.

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The special technical feature of Group XXXXI is a process for screening for and identifying an antifungal agent in *S. cerevisiae*. This feature defines an advance over Group I in that it is a method of use of a polypeptide that is substantially different in sequence from the isolated polypeptide claimed in Group I.

The special technical feature of Group XXXXII is a process for screening for and identifying an antifungal agent in *C. albicans*. This feature defines an advance over Group I in that it is a method of use of a polypeptide that is substantially different in sequence from the isolated polypeptide claimed in Group I.

The special technical feature of Group XXXXIII is an antifungal agent. This feature defines an advance over Group I in that it is a molecule that is substantially different in function from that of the polypeptide claimed in Group I.

Continuation of B. FIELDS SEARCHED Item 3:

SEQUENCE SEARCH, MEDLINE, WEST

Search terms: SEQ ID NO:2, fungal, fungi, fungus, yeast, polypeptide, fusion, GST, extract, Bot1