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Canadian Intellectual Property Office

CA 2833660 C 2020/08/04

(11)(21) 2 833 660

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 2012/04/20
(87) Date publication PCT/PCT Publication Date: 2012/10/26
(45) Date de délivrance/Issue Date: 2020/08/04
(85) Entrée phase nationale/National Entry: 2013/10/18
(86) N° demande PCT/PCT Application No.: US 2012/034379
(87) N° publication PCT/PCT Publication No.: 2012/145584
(30) Priorités/Priorities: 2011/04/22 (US61/478,160); 2011/04/22 (US61/478,162)

(51) Cl.Int./Int.Cl. *C12N 1/14* (2006.01), *C07K 14/37* (2006.01), *C12N 1/15* (2006.01), *C12N 15/01* (2006.01), *C12N 15/31* (2006.01), *C12P 21/00* (2006.01)

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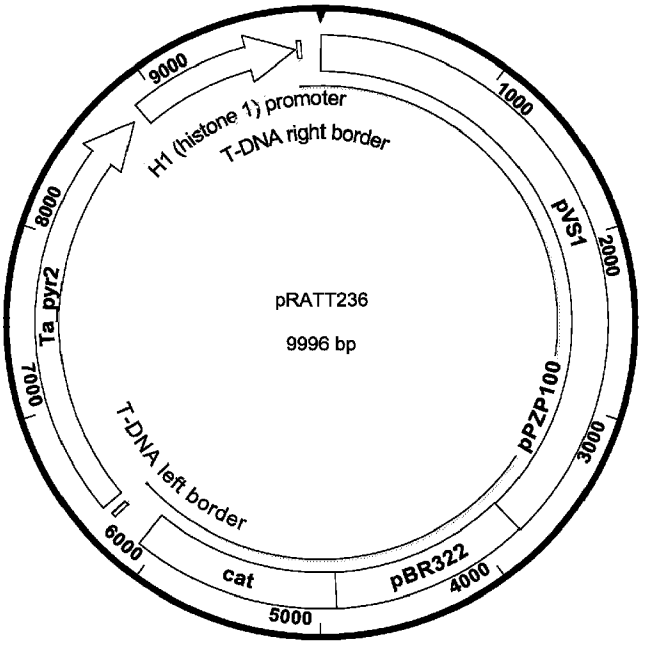
(54) Titre : CHAMPIGNONS FILAMENTEUX PRESENTANT UN PHENOTYPE DE VISCOSITE MODIFIE
(54) Title: FILAMENTOUS FUNGI HAVING AN ALTERED VISCOSITY PHENOTYPE



(57) Abrégé/Abstract:
Described are compositions and methods relating to variant filamentous fungi having altered growth characteristics. Such variants are well-suited for growth in submerged cultures, e.g., for the large-scale production of enzymes and other proteins for commercial applications.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 October 2012 (26.10.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/145584 A1

(51) **International Patent Classification**:
C07K 14/37 (2006.01) *C12N 15/01* (2006.01)
C12N 1/14 (2006.01)

(21) **International Application Number**:
PCT/US2012/034379

(22) **International Filing Date**:
20 April 2012 (20.04.2012)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
61/478,162 22 April 2011 (22.04.2011) US
61/478,160 22 April 2011 (22.04.2011) US

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

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,

[Continued on next page]

(54) **Title**: FILAMENTOUS FUNGI HAVING AN ALTERED VISCOSITY PHENOTYPE

(57) **Abstract**: Described are compositions and methods relating to variant filamentous fungi having altered growth characteristics. Such variants are well-suited for growth in submerged cultures, e.g., for the large-scale production of enzymes and other proteins for commercial applications.

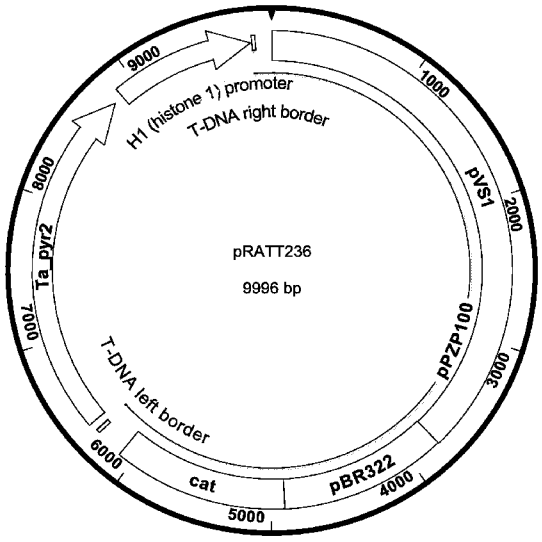

Figure 1

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LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

FILAMENTOUS FUNGI HAVING AN ALTERED VISCOSITY PHENOTYPE

PRIORITY

[001] The present application claims priority to U.S. Provisional Application Serial Nos. 61/478,162, and 61/478,160, both filed on April 22, 2011.

TECHNICAL FIELD

[002] The present strains and methods relate to genetic mutations in filamentous fungi that give rise to strain variants having altered growth characteristics. Such variants are well-suited for growth in submerged cultures, *e.g.*, for the large-scale production of enzymes and other proteins or metabolites for commercial applications.

BACKGROUND

[003] Filamentous fungi are capable of expressing native and heterologous proteins to high levels, making them well-suited for the large-scale production of enzymes and other proteins for industrial, pharmaceutical, animal health and food and beverage applications. Filamentous fungi are typically grown in mycelial submerged cultures in bioreactors, which are adapted to introduce and distribute oxygen and nutrients into the culture medium (*i.e.*, broth). The morphological characteristics of the mycelium affect the rheological properties of the broth, thereby affecting bioreactor performance.

[004] Generally, the higher the viscosity of the broth, the less uniform the distribution of oxygen and nutrients and the more energy required to agitate the culture. In some cases, the viscosity of the broth becomes sufficiently high to significantly interfere with the dissolution of oxygen and nutrients, thereby adversely affecting the growth of the fungi. Additionally, the power required to mix and aerate viscous broth can significantly increase the cost of production, and incur higher capital expenditures in terms of motors and power supplies.

SUMMARY

[005] Described are strains and methods relating to filamentous fungi having genetic alterations that give rise to altered viscosity phenotypes.

[006] In one aspect, a variant strain of filamentous fungus derived from a parental strain is provided, the variant strain comprising a genetic alteration that causes cells of the variant strain

to produce an altered amount of functional Mpg1 protein compared to cells of the parental strain, wherein the cells of the variant strain are produced during aerobic fermentation in submerged culture cell broth that (i) requires an altered amount of agitation to maintain a preselected dissolved oxygen content compared to the cells of the parental strain, and/or (ii) maintains an altered dissolved oxygen content at a preselected amount of agitation, compared to the cells of the parental strain.

[007] In some embodiments, the altered amount of functional Mpg1 protein is a reduced amount, and the variant strain produces during aerobic fermentation in submerged culture a cell broth that (i) requires reduced agitation to maintain a preselected dissolved oxygen content compared to the cells of the parental strain, and/or (ii) maintains an increased dissolved oxygen content at a preselected amount of agitation, compared to the cells of the parental strain.

[008] In some embodiments, the genetic alteration comprises a disruption of the *mpg1* gene present in the parental strain. In some embodiments, disruption of the *mpg1* gene is the result of deletion of all or part of the *mpg1* gene. In some embodiments, disruption of the *mpg1* gene is the result of deletion of a portion of genomic DNA comprising the *mpg1* gene. In some embodiments, disruption of the *mpg1* gene is the result of mutagenesis of the *mpg1* gene.

[009] In some embodiments, disruption of the *mpg1* gene is performed using site-specific recombination. In some embodiments, disruption of the *mpg1* gene is performed in combination with introducing a selectable marker at the genetic locus of the *mpg1* gene.

[010] In some embodiments, the variant strain does not produce functional Mpg1 protein. In some embodiments, the variant strain does not produce Mpg1 protein.

[011] In some embodiments, the variant strain further comprises a gene encoding a protein of interest. In some embodiments, the variant strain further comprises a disruption of the *sfb3* gene. In some embodiments, the variant strain further comprises a disruption of the *seb1*gene. In some embodiments, the variant strain further comprises a disruption of the *sfb3* and *seb1* genes. In some embodiments, the variant strain further comprises a disruption of at least one gene selected from the group consisting of the *sfb3* gene, the *seb1* gene, the *gas1* gene, the *crz1* gene, and the *tps2* gene. In some embodiments, the variant strain produces substantially the same amount of, or more, protein per unit amount of biomass as the parental strain.

[012] In some embodiments, the filamentous fungus is a Pezizomycotina species. In some embodiments, the filamentous fungus is a *Trichoderma* spp., *Aspergillus* spp., *Fusarium* spp., *Scedosporium* spp., *Penicillium* spp., *Chrysosporium* spp., *Cephalosporium* spp., *Talaromyces* spp., *Geosmithia* spp., and *Neurospora* spp. In some embodiments, the filamentous fungus can include, but is not limited to, *Trichoderma reesei* (previously classified as *Trichoderma*

longibrachiatum and *Hypocrea jecorina*), *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus itaconicus*, *Aspergillus oryzae*, *Aspergillus nidulans*, *Aspergillus terreus*, *Aspergillus sojae*, *Aspergillus japonicus*, *Scedosporium prolificans*, *Neurospora crassa*, *Penicillium funiculosum*, *Penicillium chrysogenum*, *Talaromyces (Geosmithia) emersonii*, *Fusarium venenatum*, and *Chrysosporium lucknowense*. In some embodiments, the filamentous fungus is *Trichoderma reesei*.

[013] In another aspect, a method for producing a variant strain of filamentous fungus cells is provided, comprising: introducing a genetic alteration into a parental strain of filamentous fungal cell, which genetic alteration alters the production of functional Mpg1 protein compared to the cells of the parental strain, thereby producing a variant filamentous fungal cell that produces during aerobic fermentation in submerged culture a cell broth that (i) requires an altered amount of agitation to maintain a preselected dissolved oxygen content, compared to the cells of the parental strain, and/or (ii) maintains an altered dissolved oxygen content at a preselected amount of agitation, compared to the cells of the parental strain.

[014] In some embodiments, the genetic alteration reduces or prevents the production of functional Mpg1 protein, thereby producing a variant filamentous fungal cell that produces during aerobic fermentation in submerged culture a cell broth that (i) requires reduced agitation to maintain a preselected dissolved oxygen content, compared to the cells of the parental strain, and/or (ii) maintains an increased dissolved oxygen content at a preselected amount of agitation, compared to the cells of the parental strain.

[015] In some embodiments, the genetic alteration comprises disrupting the *mpg1*gene in a parental filamentous fungal cell using genetic manipulation. In some embodiments, the genetic alteration comprises deleting the *mpg1* gene in a parental filamentous fungal cell using genetic manipulation. In some embodiments, the genetic alteration is performed using site-specific genetic recombination.

[016] In some embodiments, disruption of the *mpg1* gene is performed in combination with introducing a selectable marker at the genetic locus of the *mpg1* gene. In some embodiments, disruption of the *mpg1* gene is performed in combination with disrupting the *sfb3* gene. In some embodiments, disruption of the *mpg1* gene is performed in combination with disrupting at least one gene selected from the group consisting of the *sfb3* gene, the *seb1* gene, the *gas1* gene, the *crz1* gene, and the *tps2* gene.

[017] In some embodiments, the variant strain produces substantially the same amount of, or more, protein per unit amount of biomass as the parental strain.

[018] In some embodiments, the filamentous fungus is a Pezizomycotina species. In some embodiments, the filamentous fungus is a *Trichoderma* spp., *Aspergillus* spp., *Fusarium* spp., *Scedosporium* spp., *Penicillium* spp., *Chrysosporium* spp., *Cephalosporium* spp., *Talaromyces* spp., *Geosmithia* spp., and *Neurospora* spp. In some embodiments, the filamentous fungus can include, but is not limited to, *Trichoderma reesei* (previously classified as *Trichoderma longibrachiatum* and *Hypocrea jecorina*), *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus itaconicus*, *Aspergillus oryzae*, *Aspergillus nidulans*, *Aspergillus terreus*, *Aspergillus sojae*, *Aspergillus japonicus*, *Scedosporium prolificans*, *Neurospora crassa*, *Penicillium funiculosum*, *Penicillium chrysogenum*, *Talaromyces (Geosmithia) emersonii*, *Fusarium venenatum*, and *Chrysosporium lucknowense*. In some embodiments, the filamentous fungus is *Trichoderma reesei*.

[019] In some embodiments, the parental strain further comprises a gene encoding a protein of interest. In some embodiments, the gene encoding the protein of interest is present in the parental strain prior to introducing the genetic alteration that reduces or prevents the production of functional Mpg1 protein. In some embodiments the protein of interest within the parental strain is encoded by an endogenous gene or a heterologous gene.

[020] In another aspect, a protein of interest produced by any of the aforementioned variant strains is provided.

[021] In yet another aspect, a filamentous fungus produced by any of the aforementioned methods and having any of the aforementioned properties is provided.

[022] In another aspect, a variant strain of filamentous fungus derived from a parental strain is provided, the variant strain comprising: (a) a genetic alteration that results in (i) a requirement for reduced agitation in submerged culture to maintain a preselected dissolved oxygen content, compared to the cells of the parental strain, and/or (ii) maintenance of an increased dissolved oxygen content in submerged culture at a preselected amount of agitation, compared to the cells of the parental strain, and (b) a gene encoding a protein of interest, wherein the gene encoding the protein of interest is present in the variant strain prior to the genetic alteration in (a).

[023] In some embodiments, the genetic alteration of the resulting variant strain comprises a disruption of the *mpg1* gene present in the parental strain. In some embodiments, disruption of the *mpg1* gene is performed in combination with introducing a selectable marker at the genetic locus of the *mpg1* gene. In some embodiments, disruption of the *mpg1* gene is performed in combination with disrupting the *sfb3* gene. In some embodiments, disruption of the *mpg1* gene is performed in combination with disrupting the *seb1* gene. In some embodiments, disruption of

the *mpg1* gene is performed in combination with disrupting at least one gene selected from the group consisting of the *sfb3* gene, the *seb1* gene, the *gas1* gene, the *crz1* gene, and the *tps2* gene.

[024] These and other aspects and embodiments of present variant strains and methods will be apparent from the description, including the accompanying Figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[025] Figure 1 is a map of the *Agrobacterium tumefaciens* pRATT 236 vector.

[026] Figure 2 is a map of the *mpg1* disruption vector.

[027] Figure 3 is a map of the *seb1* disruption vector.

DETAILED DESCRIPTION

I. Overview

[028] The present strains and methods relate to variant strains of filamentous fungus cells having genetic modifications that affect their morphology and growth characteristics. When the variant cells are grown in submerged culture, they produce a cell broth that has different rheological properties compared to a cell broth comprising cells of the parental strain. Some of these variant strains are well-suited for the large-scale production of enzymes and other commercially important proteins.

II. Definitions

[029] Prior to describing the present strains and methods in detail, the following terms are defined for clarity. Terms not defined should be accorded their ordinary meanings as used in the relevant art.

[030] As used herein, "*Trichoderma reesei*" refers to a filamentous fungus of the phylum Ascomycota, subphylum Pezizomycotina. This organism was previously classified as *Trichoderma longibrachiatum*, and also as *Hypocrea jecorina.*

[031] As used herein, the phrase "variant strain of filamentous fungus cells," or similar phrases, refer to strains of filamentous fungus cells that are derived (*i.e.*, obtained from or obtainable from) from a parental (or reference) strain belonging to the Pezizomycotina, *e.g.*, by genetic manipulation. In the present description, parental and variant strains can be described as having certain characteristics, such as genetic modifications, expression phenotypes, morphology, and the like; however, the skilled person will appreciate that it is technically the

cells of the parental or variant strain that have such characteristics, and "the strains" are referred to for convenience.

[032] As used herein, the term "protein of interest" refers to a polypeptide that is desired to be expressed in a filamentous fungus. Such a protein can be an enzyme, a substrate-binding protein, a surface-active protein, a structural protein, or the like, and can be expressed at high levels, and can be for the purpose of commercialization. The protein of interest can be encoded by an endogenous gene or a heterologous gene relative to the variant strain and/or the parental strain. The protein of interest can be expressed intracellularly or as a secreted protein.

[033] As used herein, the phrase "substantially free of an activity," or similar phrases, means that a specified activity is either undetectable in an admixture or present in an amount that would not interfere with the intended purpose of the admixture.

[034] As used herein, the terms "polypeptide" and "protein" (and/or their respective plural forms) are used interchangeably to refer to polymers of any length comprising amino acid residues linked by peptide bonds. The conventional one-letter or three-letter codes for amino acid residues are used herein. The polymer can be linear or branched, it can comprise modified amino acids, and it can be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as well as other modifications known in the art.

[035] As used herein, functionally and/or structurally similar proteins are considered to be "related proteins." Such proteins can be derived from organisms of different genera and/or species, or even different classes of organisms (*e.g.*, bacteria and fungi). Related proteins also encompass homologs determined by primary sequence analysis, determined by secondary or tertiary structure analysis, or determined by immunological cross-reactivity.

[036] As used herein, the term "derivative polypeptide/protein" refers to a protein which is derived or derivable from a protein by addition of one or more amino acids to either or both the N- and C-terminal end(s), substitution of one or more amino acids at one or a number of different sites in the amino acid sequence, deletion of one or more amino acids at either or both ends of the protein or at one or more sites in the amino acid sequence, and/or insertion of one or more amino acids at one or more sites in the amino acid sequence. The preparation of a protein derivative can be achieved by modifying a DNA sequence which encodes for the native protein,

transformation of that DNA sequence into a suitable host, and expression of the modified DNA sequence to form the derivative protein.

[037] Related (and derivative) proteins include "variant proteins." Variant proteins differ from a reference/parental protein (*e.g.*, a wild-type protein) by substitutions, deletions, and/or insertions at a small number of amino acid residues. The number of differing amino acid residues between the variant and parental protein can be one or more, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, or more amino acid residues. Variant proteins can share at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or even at least about 99%, or more, amino acid sequence identity with a reference protein. A variant protein can also differ from a reference protein in selected motifs, domains, epitopes, conserved regions, and the like.

[038] As used herein, the term "analogous sequence" refers to a sequence within a protein that provides similar function, tertiary structure, and/or conserved residues as the protein of interest (*i.e.*, typically the original protein of interest). For example, in epitope regions that contain an α-helix or a β-sheet structure, the replacement amino acids in the analogous sequence preferably maintain the same specific structure. The term also refers to nucleotide sequences, as well as amino acid sequences. In some embodiments, analogous sequences are developed such that the replacement amino acids result in a variant enzyme showing a similar or improved function. In some embodiments, the tertiary structure and/or conserved residues of the amino acids in the protein of interest are located at or near the segment or fragment of interest. Thus, where the segment or fragment of interest contains, for example, an α-helix or a β-sheet structure, the replacement amino acids preferably maintain that specific structure.

[039] As used herein, the term "homologous protein" refers to a protein that has similar activity and/or structure to a reference protein. It is not intended that homologs necessarily be evolutionarily related. Thus, it is intended that the term encompass the same, similar, or corresponding enzyme(s) (*i.e.*, in terms of structure and function) obtained from different organisms. In some embodiments, it is desirable to identify a homolog that has a quaternary, tertiary and/or primary structure similar to the reference protein. In some embodiments, homologous proteins induce similar immunological response(s) as a reference protein. In some embodiments, homologous proteins are engineered to produce enzymes with desired activity(ies).

[040] The degree of homology between sequences can be determined using any suitable method known in the art (see, *e.g.*, Smith and Waterman (1981) *Adv. Appl. Math.* 2:482;

Needleman and Wunsch (1970) *J. Mol. Biol.*, 48:443; Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. USA* 85:2444; programs such as GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package (Genetics Computer Group, Madison, WI); and Devereux *et al.* (1984) *Nucleic Acids Res.* 12:387-95).

[041] For example, PILEUP is a useful program to determine sequence homology levels. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pair-wise alignments. It can also plot a tree showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng and Doolittle, (Feng and Doolittle (1987) *J. Mol. Evol.* 35:351-60). The method is similar to that described by Higgins and Sharp ((1989) *CABIOS* 5:151-53). Useful PILEUP parameters including a default gap weight of 3.00, a default gap length weight of 0.10, and weighted end gaps. Another example of a useful algorithm is the BLAST algorithm, described by Altschul *et al.* ((1990) *J. Mol. Biol.* 215:403-10) and Karlin *et al.* ((1993) *Proc. Natl. Acad. Sci. USA* 90:5873-87). One particularly useful BLAST program is the WU-BLAST-2 program (see, *e.g.*, Altschul *et al.* (1996) *Meth. Enzymol.* 266:460-80). Parameters "W," "T," and "X" determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word-length (W) of 11, the BLOSUM62 scoring matrix (see, *e.g.*, Henikoff and Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915) alignments (B) of 50, expectation (E) of 10, M′5, N′-4, and a comparison of both strands.

[042] As used herein, the phrases "substantially similar" and "substantially identical," in the context of at least two nucleic acids or polypeptides, typically means that a polynucleotide or polypeptide comprises a sequence that has at least about 70% identity, at least about 75% identity, at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, or even at least about 99% identity, or more, compared to the reference (*i.e.*, wild-type) sequence. Sequence identity can be determined using known programs such as BLAST, ALIGN, and CLUSTAL using standard parameters. (See, *e.g.*, Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-410; Henikoff *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 89:10915; Karin *et al.* (1993) *Proc. Natl. Acad. Sci USA* 90:5873; and Higgins *et al.* (1988) *Gene* 73:237-244). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. Also, databases can be searched using FASTA (Pearson *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:2444-48). One indication that two polypeptides are substantially identical is that the first polypeptide is immunologically cross-

reactive with the second polypeptide. Typically, polypeptides that differ by conservative amino acid substitutions are immunologically cross-reactive. Thus, a polypeptide is substantially identical to a second polypeptide, for example, where the two peptides differ only by a conservative substitution. Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions (*e.g.*, within a range of medium to high stringency).

[043] As used herein, the term "gene" is synonymous with the term "allele" in referring to a nucleic acid that encodes and directs the expression of a protein or RNA. Vegetative forms of filamentous fungi are generally haploid, therefore a single copy of a specified gene (*i.e.*, a single allele) is sufficient to confer a specified phenotype.

[044] As used herein, the terms "wild-type" and "native" are used interchangeably and refer to genes, proteins, or strains, found in nature.

[045] As used herein, "deletion of a gene," refers to its removal from the genome of a host cell. Where a gene includes control elements (*e.g.*, enhancer elements) that are not located immediately adjacent to the coding sequence of a gene, deletion of a gene refers to the deletion of the coding sequence, and optionally adjacent enhancer elements, including but not limited to, for example, promoter and/or terminator sequences.

[046] As used herein, "disruption of a gene" refers broadly to any genetic or chemical manipulation, *i.e.*, mutation, that substantially prevents a cell from producing a function gene product, *e.g.*, a protein, in a host cell. Exemplary methods of disruption include complete or partial deletion of any portion of a gene, including a polypeptide-coding sequence, a promoter, an enhancer, or another regulatory element, or mutagenesis of the same, where mutagenesis encompasses substitutions, insertions, deletions, inversions, and combinations and variations, thereof, any of which mutations substantially prevent the production of a function gene product. A gene can also be disrupted using RNAi, antisense, or any other method that abolishes gene expression.

[047] As used herein, the terms "genetic manipulation" and "genetic alteration" are used interchangeably and refer to the alteration/change of a nucleic acid sequence. The alteration can included but is not limited to a substitution, deletion, insertion or chemical modification of at least one nucleic acid in the nucleic acid sequence.

[048] As used herein, "aerobic fermentation" refers to growth in the presence of oxygen.

[049] As used herein, the term "cell broth" refers collectively to medium and cells in a liquid/submerged culture.

[050] As used herein, the term "cell mass" refers to the cell component (including intact and lysed cells) present in a liquid/submerged culture. Cell mass can be expressed in dry or wet weight.

[051] As used herein, the term "rheology" refers to a branch of physics dealing with the deformation and flow of matter.

[052] As used herein, "viscosity" is a measure of the resistance of a fluid to deformation by mechanical stress, such as shear stress or tensile stress. In the present context, viscosity can also refer to the resistance of a cell broth comprising filamentous fungus cells to mechanical stress, *e.g.*, as provided by a rotor/impeller. Because the viscosity of a cell broth can be difficult to measure directly, indirect measurements of viscosity can be used, such as the dissolved oxygen content of the culture broth at a preselected amount of agitation, the amount of agitation required to maintain a preselected dissolved oxygen content, the amount of power required to agitate a cell broth to maintain a preselected dissolved oxygen content, or even colony morphology on solid medium.

[053] As used herein, an "altered-viscosity" variant strain of filamentous fungus cells refers to a variant strain that produces a cell broth that has either a reduced or increased viscosity (*i.e.*, reduced or increased resistance to shear or tensile stress) compared to an equivalent cell broth produced by a parental strain. Generally, equivalent cell broths have comparable cell masses. Preferably, the difference between a variant, altered viscosity strain and a parental strain, with respect to any direct or indirect measure of viscosity, is at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or even at least 50%, or more. Methods for comparing the viscosity of filamentous fungus cell broths are described, herein. Generally, comparable (or equivalent) cell broths have comparable cell masses.

[054] As used herein, a "reduced-viscosity" variant strain of filamentous fungus cells refers to a variant strain that produces a cell broth that has reduced viscosity (*i.e.*, reduced resistance to shear or tensile stress) compared to an equivalent cell broth produced by a parental strain. Preferably, the difference between a variant, altered viscosity strain and a parental strain, with respect to any direct or indirect measure of viscosity, is at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or even at least 50%, or more.

[055] As used herein, "dissolved oxygen" (DO) refers to the amount of oxygen (O_2) present in a liquid medium as measured in vol/vol units. The dissolved oxygen level can be maintained at a high level, *e.g.,* between 170-100% and 20%, between 100-80% and 20%, between 70% and 20%, between 65% and 20%, between 60% and 20%, between 55% and 20%, between 50% and

20%, between 45% and 20%, between 44% and 20%, between 43% and 20%, between 42% and 20%, between 41% and 20%, between 40% and 20%, between 35% and 20%, between 30% and 20%, and between 25% and 20% throughout the fermentation. In particular, the dissolved oxygen can be high at the beginning of the fermentation and to be permitted to fall as the fermentation progresses. The dissolved oxygen level can be controlled by the rate at which the fermentation is agitated, e.g. stirred, and/or by the rate of addition of air or oxygen. The culture can be agitated, *e.g.,* stirred at between 400-700 rpm and the dissolved oxygen level is maintained above 20%, above 25%, above 30%, above 35%, above 40%, above 45%, above 50% and above 55% or more by altering the air or oxygen flow rate and impeller speed.

[056] As used herein, a "primarily genetic determinant" refers to a gene, or genetic manipulation thereof, that is necessary and sufficient to confer a specified phenotype in the absence of other genes, or genetic manipulations, thereof. However, that a particular gene is necessary and sufficient to confer a specified phenotype does not exclude the possibility that additional effects to the phenotype can be achieved by further genetic manipulations.

[057] As used herein, a "functional polypeptide/protein" is a protein that possesses an activity, such as an enzymatic activity, a binding activity, a surface-active property, or the like, and which has not been mutagenized, truncated, or otherwise modified to abolish or reduce that activity. Functional polypeptides can be thermostable or thermolabile, as specified.

[058] As used herein, "a functional gene" is a gene capable of being used by cellular components to produce an active gene product, typically a protein. Functional genes are the antithesis of disrupted genes, which are modified such that they cannot be used by cellular components to produce an active gene product, or have a reduced ability to be used by cellular components to produce an active gene product.

[059] As used herein, variant cells "maintain or retain a high level of protein expression and/or secretion" compared to a parental strain if the difference in protein expression between the variant strain and a parental strain is less than about 20%, less than about 15%, less than about 10%, less than about 7%, less than about 5%, or even less than about 3%.

[060] As used herein, host cells have been "modified to prevent the production of a specified protein" if they have been genetically or chemically altered to prevent the production of a functional protein/polypeptide that exhibits an activity characteristic of the wild-type protein, particularly an activity that promotes elongation of hyphae or otherwise increases the viscosity of a filamentous fungus in liquid culture. Such modifications include, but are not limited to, deletion or disruption of the gene encoding the protein (as described herein), modification of the

gene such that the encoded polypeptide lacks the aforementioned activity, modification of the gene to affect post-translational processing or stability, and combinations, thereof.

[061] As used herein, a "protein of interest" is a protein that is desired to be produced in a submerged culture of filamentous fungus cells. Generally, proteins of interest are commercially important for industrial, pharmaceutical, animal health, and food and beverage use, making them desirable to produce in large quantities. Proteins of interest are to be distinguished from the myriad other proteins expressed by the filamentous fungus cells, which are generally not of interest as products and are mainly considered background protein contaminants.

[062] As used herein, a variant strain produces "substantially the same amount" of protein per unit amount of biomass as a parental strain if the amount of protein produced by the variant strain is no more than 20% reduced, no more than 15% reduced, no more than 10% reduced, an even no more than 5% reduced compared to the amount of protein produced by the parental strain, wherein the amount of protein is normalized to the total amount of biomass of cells from which protein production is measured, wherein biomass can be expressed in terms of either wet (*e.g.*, of cell pellet) or dry weight.

[063] As used herein, a variant strain produces "substantially more protein per unit amount of biomass" than a parental strain if the amount of protein produced by the variant strain is at least 5% increased, at least 10% increased, at least 15% increased, or more, compared to the parental strain, wherein the amount of protein is normalized to the total amount of biomass of cells from which protein production is measured, wherein biomass can be expressed in terms of either wet (*e.g.*, of cell pellet) or dry weight.

[064] As used herein, "fluorochromes" are fluorescent dyes. Preferred fluorochromes bind to cellulose and/or chitin in the cell walls of fungi.

[065] As used herein, the singular articles "a," "an," and "the" encompass the plural referents unless the context clearly dictates otherwise.

The following abbreviations/acronyms have the following meanings unless otherwise specified:

CFU	colony forming units
EC	enzyme commission
kDa	kiloDalton
kb	kilobase
MW	molecular weight
w/v	weight/volume
w/w	weight/weight
v/v	volume/volume
wt%	weight percent
°C	degrees Centigrade

H_2O	water
H_2O_2	hydrogen peroxide
dH_2O or DI	deionized water
dIH_2O	deionized water, Milli-Q filtration
DO	dissolved oxygen
g or gm	gram
µg	microgram
mg	milligram
kg	kilogram
lb	pound
µL and µl	microliter
mL and ml	milliliter
mm	millimeter
µm	micrometer
mol	mole
mmol	millimole
M	molar
mM	millimolar
µM	micromolar
nm	nanometer
U	unit
ppm	parts per million
sec and "	second
min and '	minute
hr and h	hour
EtOH	ethanol
eq.	equivalent
N	normal
PCR	polymerase chain reaction
DNA	deoxyribonucleic acid
FOA	fluoroorotic acid
UV	ultraviolet
A_{540}	absorbance measured at a wavelength of 540 nm
CMC	carboxymethyl cellulose
rpm	revolutions per minute
Δ	relating to a deletion
CER	CO_2 evolution rate
bp	base pairs

III. Filamentous fungal strain with altered Mpg1 protein production

[066] In one aspect, a variant strain of filamentous fungus derived from a parental strain is provided, the variant strain comprising a genetic alteration that causes cells of the variant strain to produce an altered amount of functional Mpg1 protein compared to cells of the parental strain. The cells of the variant strain subsequently produce, during aerobic fermentation in submerged culture, a cell broth that requires an altered amount of agitation to maintain a preselected dissolved oxygen content, or a cell mass that maintains an altered dissolved oxygen content at a preselected amount of agitation, compared to the cells of the parental strain.

[067] In some cases, the genetic alteration causes cells of the variant strain to produce a reduced amount of functional Mpg1 protein compared to cells of the parental strain, and the resulting cell broth requires reduced agitation to maintain a preselected dissolved oxygen content or maintains a higher dissolved oxygen content at a preselected amount of agitation compared to the cells of the parental strain. In such cases, it is believed that the cell mass of the variant strain exhibits reduced viscosity compared to a cell mass of the parental strain, which accounts for the observations relating to dissolved oxygen content and agitation, as described in the Examples.

[068] The reduction in the amount of functional Mpg1 protein can result from disruption of the *mpg1* gene present in the parental strain. Because disruption of the *mpg1* gene is a primary genetic determinant for conferring a reduced viscosity phenotype to the variant strain, such variant strains need only comprise a disrupted *mpg1* gene, while all other genes can remain intact. In some cases, the variant strains can optionally include additional genetic alterations compared to the parental stain from which they are derived. Such additional genetic alterations are not necessary to confer a reduction in viscosity but can further reduce viscosity or confer other advantages for the variant strain.

[069] Disruption of the *mpg1* gene can be performed using any suitable methods that substantially prevent expression of a function *mpg1* gene product, *i.e.*, the Mpg 1 protein. Exemplary methods of disruption as are known to one of skill in the art include but are not limited to: Complete or partial deletion of the *mpg1* gene, including complete or partial deletion of, *e.g.*, the Mpg1-coding sequence, the promoter, the terminator, an enhancer, or another regulatory element; and complete or partial deletion of a portion of the chromosome that includes any portion of the *mpg1* gene. Particular methods of disrupting the *mpg1* gene include making nucleotide substitutions or insertions in any portion of the *mpg1* gene, *e.g.*, the Mpg1-coding sequence, the promoter, the terminator, an enhancer, or another regulatory element. Preferably, deletions, insertions, and/or substitutions (collectively referred to as mutations) are made by genetic manipulation using sequence-specific molecular biology techniques, as opposed to by chemical mutagenesis, which is generally not targeted to specific nucleic acid sequences. Nonetheless, chemical mutagenesis can be used to disrupt the *mpg1* gene.

[070] Mutations in the *seb1* gene can reduce the efficiency of the *mpg1* promoter, reduce the efficiency of a *mpg1* enhancer, interfere with the splicing or editing of the *mpg1* mRNA, interfere with the translation of the *mpg1* mRNA, introduce a stop codon into the Mpg1-coding sequence to prevent the translation of full-length Mpg1 protein, change the coding sequence of the Mpg1 protein to produce a less active or inactive protein or reduce Mpg1 interaction with other cell wall components, change the coding sequence of the Mpg1 protein to produce a less stable

protein or target the protein for destruction, cause the Mpg1 protein to misfold or be incorrectly modified (*e.g.*, by glycosylation), or interfere with cellular trafficking of the Mpg1 protein.

[071] In one embodiment, these and other genetic manipulations act to reduce or prevent the expression of a functional Mpg1 protein, or reduce or prevent the normal biological activity of the Mpg1 protein, thereby producing a morphology change in the cell that results in a reduced viscosity phenotype.

[072] In other cases, the genetic alteration increases or restores the expression of a functional Mpg1 protein, or increases the normal biological activity of the Mpg1 protein, thereby producing a morphology change in the cell that results in an increased or restored viscosity phenotype. Exemplary genetic alterations that increase or restore Mpg1 function are those that introduce addition copies of the *mpg1* gene into a cell, increase the efficiency of the *mpg1* promoter, enhancer, or other control element, increase the translation of the mRNA encoding the Mpg1 protein, increase the stability of mRNA encoding the Mpg1 protein, introduce changes in the *mpg1* gene that increase the activity or stability of the Mpg1 protein, introduce changes in the *mpg1* gene that modulate the interaction with other proteins or cell wall components, and the like. Other genetic alterations that increase or restore Mpg1 function are those that reverse the effect of genetic alterations that reduce or prevent the expression of a functional Mpg1 protein

[073] Filamentous fungus cells for manipulation and use as described are generally from the phylum Ascomycota, subphylum Pezizomycotina, particularly fungi that have a vegetative hyphae state and include a homolog of the *mpg1* gene. Such organisms include filamentous fungus cells used for the production of commercially important industrial and pharmaceutical proteins, including, but are not limited to *Trichoderma* spp., *Aspergillus* spp., *Fusarium* spp., *Scedosporium* spp., *Penicillium* spp., *Chrysosporium* spp., *Cephalosporium* spp., *Talaromyces* spp., *Geosmithia* spp., and *Neurospora* spp. Particular organisms include, but are not limited to, *Trichoderma reesei* (previously classified as *Trichoderma longibrachiatum* and *Hypocrea jecorina*), *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus itaconicus*, *Aspergillus oryzae*, *Aspergillus nidulans*, *Aspergillus terreus*, *Aspergillus sojae*, *Aspergillus japonicus*, *Scedosporium prolificans*, *Neurospora crassa*, *Penicillium funiculosum*, *Penicillium chrysogenum*, *Talaromyces (Geosmithia) emersonii*, *Fusarium venenatum*, and *Chrysosporium lucknowense*.

[074] As described by Kruszewska *et al.* (1998) *Cur. Genet.* 33:445-50 and Zakrzewska *et al.* (2003) *Applied and Environmental Microbiology* 69:4383-89), Mpg 1(PID 122551) from *Trichoderma reesei* encodes a GTP:alpha-D-mannose-1-phoshate guanyltransferase. Over-expression of the *mpg1* gene increases GDP-mannose levels, which can play a major regulatory

role in early stages of protein glycosylation. However, Mpg1 has heretofore not been described previously as being associated with altered morphology, particularly not an altered morphology that gives rise to a low viscosity phenotype. The present disclosure provides experimental evidence of the association of Mpg1 with altered morphology.

[075] The amino acid sequence of the *Trichoderma reesei* Mpg 1 ((jgi|Trire2|122551) protein is shown, below, as SEQ ID NO: 1:

MKGLILVGGFGTRLRPLTLTLPKPLVEFCNKPMIVHQIEALVAAGVTDIVLAVNYRPEIMEKFL
AEYEEKYNINIEFSVESEPLDTAGPLKLAERILGKDDSPFFVLNSDVICDYPFKELLEFHKAHG
DEGTIVVTKVEEPSKYGVVVHKPNHPSRIDRFVEKPVEFVGNRINAGMYIFNPSVLKRIELRPT
SIEKETFPAMVADNQLHSFDLEGFWMDVGQPKDFLSGTCLYLSSLTKKGSKELTPPTEPYVHGG
NVMIHPSAKIGKNCRIGPNVTIGPDVVVGDGVRLQRCVLLKGSKVKDHAWVKSTIVGWNSTVGR
WARLENVTVLGDDVTIGDEIYVNGGSVLPHKSIKANVDVPAIIM

[076] The amino acid sequence of the *Neurospora crassa* Mpg1 protein is shown, below, as SEQ ID NO: 2:

MKALILVGGFGTRLRPLTLTMPKPLVEFGNKRMILHQIEALAAAGVTDIVLAVNYRPEIMEKYL
AEYEKQFGINITISIESEPLGTAGPLKLAEDVLRKDDTPFFVLNSDVTCEYPFKELAAFHKAHG
DEGTIVVTKVEEPSKYGVVVHKPNHPSRIDRFVEKPVQFVGNRINAGLYIFNPSVIDRVELRPT
SIEQETFPAMVRDGQLHSFDLEGFWMDIGQPKDFLTGTCLYLSSLTKKGSKELAPTTLPYIHGG
NVLIDPSAKIGKNCRIGPNVTIGPNVVVGDGVRLQRCVLLEGSKVKDHAWVKSTIVGWNSTVGK
WARLENVTVLGDDVTIGDEIYVNGGSILPHKTIKANVDVPAIIM

[077] The amino acid sequence of the *Aspergillus oryzae* Mannose-1-phosphate guanyltransferase protein is shown, below, as SEQ ID NO: 3:

MKGVGGGTRRTTKVCNKMVHAVAAGVTDVAVNYRMKAYKMKAVGGGTRRTTKVGNRMHVSAAAG
VTDVAVNYRDVMVSAKKYYNNSVSDTAGKARGKDDSVNSDVCDYKHKAHGDGTVVTKVYNVKSV
SGTAGKAKGKDDSVNSDVCDYKAHKKHGDGTVVTKVDSKYGVVVHKNHSRDRVKVVGNRNAGMY
NSVKRRTSKTAMVADNHSSKYGVVVHKNHSRDRVKVVGNRNAGYMNSVNRRTSTACKDGHSDGW
MDVGKDSGTCYSSTKKGSKTTYVHGGNVMHSAKGKNCRGNVTGDGWMDVGKDSGTCYTSAKRNS
KANSYVYGGNVMVDSAKGKNCRGNVVGDVVVGDGVRRCVKGSKVKDHAWVKSTVGWNSTVGRWA
RNVTVGDDVTGDYVNGGSVHNVVVGDGVRRCVNSKVKDHAWVKSTVGWNSSVGRWARNVTVGDD
VTADVYVNGGSHKSKANVDVAMKSKNVDVAM

[078] In some embodiments of the present compositions and methods, the amino acid sequence of the Mpg1 protein that is altered in production levels has a specified degree of overall amino

acid sequence identity to the amino acid sequence of SEQ ID NOs: 1, 2, or 3, *e.g.*, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or even at least about 99% identity, to SEQ ID NOs: 1, 2, or 3. The nucleotide sequences encoding each amino acid sequence can be identified from a BLAST search for each corresponding protein as is know to one skilled in the art.

[079] In some embodiments of the present compositions and methods, the *mpg1* gene that is disrupted encodes a Mpg1 protein that has a specified degree of overall amino acid sequence identity to the amino acid sequence of SEQ ID NOs: 1, 2, or 3, *e.g.*, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or even at least about 99% identity, to SEQ ID NOs: 1, 2, or 3.

[080] The amino acid sequence information provided, herein, readily allows the skilled person to identify an Mpg1 protein, and the nucleic acid sequence encoding an Mpg1 protein, in any filamentous fungi, and to make appropriate disruptions in the *mpg1* gene to affect the production of the Mpg1 protein. The polynucleotide sequences encoding SEQ ID NOs: 1, 2 and 3 can be found in the GenBank or JGI databases, as are known to one of skill in the art.

[081] In another aspect, a method for altering the morphology of filamentous fungus cells is provided. The variant filamentous fungus cells exhibit altered growth morphology on solid medium and produce cell masses having different viscosities when grown in submerged culture compared to parental cell growth and cell broth viscosities.

[082] In some cases, the method comprises disrupting the *mpg1* gene in a parental strain using suitable genetic methods, wherein during aerobic fermentation the disrupted *mpg1* variant strain produces during aerobic fermentation in submerged culture a cell broth that requires reduced agitation to maintain a preselected dissolved oxygen content, or maintains an increased dissolved oxygen content at a preselected amount of agitation, compared to the cells of the parental strain. Such methods can be used to disrupt the *mpg1* gene in any manner described above and elsewhere as are known t one of skill in the art. Preferably, disruption of the *mpg1* gene is performed by genetic manipulation using sequence-specific molecular biology techniques, as opposed to chemical mutagenesis, which is generally not targeted to specific nucleic acid sequences. However, chemical mutagenesis can be used with satisfactory results.

[083] In some embodiments, the parental strain into which the reduced viscosity phenotype is introduced creating a reduced viscosity strain already comprises a gene of interest intended to be expressed at high levels. In this manner, the present methods obviate the need to introduce a gene of interest into a pre-existing reduced viscosity strain for production. Thus, the present methods can be used to produce a reduced viscosity variant strain of filamentous fungus cells from a parental strain already comprising a gene of interest.

IV. Additive effect produced by altering Seb1 production

[084] In some embodiments of the present compositions and methods, genetic alterations that affect Mpg1 production are combined with genetic alterations that affect Seb1 production. The *seb1*gene from *Trichoderma atroviride* is a STRE-element-binding protein, and the *seb1* gene is believed to be an orthologue of the yeast msn2/4 gene and the *Aspergillus nidulans msnA* gene. Notably, the *seb1* gene cannot complement the *msn2/4* gene in yeast, so it is probably not a functional homologue. Seb1 is involved with but not essential in the osmotic stress response but has not been described as being associated with altered morphology, particularly those giving rise to a low viscosity phenotype.

[085] A BLAST search of the publicly available genomic DNA sequence of *Trichoderma reesei* performed using the *T. atroviride* Seb1 amino acid sequence (SEQID NO: 4) as a query revealed that the *T. reesei* genome includes a single gene that is closely homologous to *seb1*. No further homologs or similar sequences were identified, suggesting that *seb1* is a unique single copy gene. Homologs of the Seb1 proteins were found in *e.g.*, *T. reesei* (SEQ ID NO: 5), *Aspergillus clavatus* (SEQ ID NO: 6), *Aspergillus fumigatus* Af93 (SEQ ID NO: 7), and *Neosartorya fischeri* NRRL 181 (SEQ ID NO: 8):

[086] The amino acid sequence of the *Trichoderma atroviride* Seb1 protein is shown, below, as SEQ ID NO: 4:

MDGMMSQAMGQQAFYFYNHNHDHKMARQAIFAQQMAAYQMVPTLPPTPMYSRPNSSCSQPPTLY
SNGPSVMTPTSTPPLSRKHMMLDAEFGDNPYFPSTPPLSTSGSTVGSPKACDMLQTPMNPMFSG
LEGIAMKEAVDTTESLVVDWASIVSPPLSPVYFQSQVSRVPSPTSSPSDILSTASCPSLSPSPT
PYARSVTSEHDVDFCDPRNLTVSVGSNPTLAPEFTLTGLAEDLKGEQLSTAQHTFDFNPALPSG
LPTFEDFSDLESEADFSNLVNLGEVNPIDISRPRACTGSSVVSLGHGSFIGDEELSFEDNDAFG
FNSLPSPTSSIDFSDVHQDKRRKKEKKDIKPIMNTAASGSPSGNEQIGATPAASAASDSNASSA
SEDPSSMPAPTNRRGRKQSLTEDPSKTFVCDLCNRRFRRQEHLKRHYRSLHTQEKPFECNECGK
KFSRSDNLAQHARTHAGGAIVMNLIEDGSEVPAFDGSMMTGPVGDDYNTYGKVLFQIASEIPGS
ASELSSEEGDQSKKKRKRSD

[087] The predicted amino acid sequence of the *Trichoderma reesei* Seb1 protein is shown, below, as SEQ ID NO: 5:

MDGMMSQPMGQQAFYFYNHEHKMSPRQVIFAQQMAAYQMMPSLPPTPMYSRPNSSCSQPPTLYS
NGPSVMTPTSTPPLSSRKPMLVDTEFGDNPYFPSTPPLSASGSTVGSPKACDMLQTPMNPMFSG
LEGIAIKDSIDATESLVLDWASIASPPLSPVYLQSQTSSGKVPSLTSSPSDMLSTTASCPSLSP
SPTPYARSVTSEHDVDFCDPRNLTVSVGSNPTLAPEFTLLADDIKGEPLPTAAQPSFDFNPALP
SGLPTFEDFSDLESEADFSSLVNLGEINPVDISRPRACTGSSVVSLGHGSFIGDEDLSFDDEAF
HFPSLPSPTSSVDFCDVHQDKRQKKDRKEAKPVMNSAAGGSQSGNEQAGATEAASAASDSNASS
ASDEPSSSMPAPTNRRGRKQSLTEDPSKTFVCDLCNRRFRRQEHLKRHYRSLHTQEKPFECNEC
GKKFSRSDNLAQHARTHSGGAIVMNLIEESSEVPAYDGSMMAGPVGDDYSTYGKVLFQIASEIP
GSASELSSEEGEQGKKKRKRSD

[088] The amino acid sequence of the *Aspergillus clavatus* Seb1 protein is shown, below, as SEQ ID NO: 6:

MDTTYTMVGTPVQGQPSFAYYTTNDSQSRQQHFTSHPSEMQAFYGQMQPYPQQQQQTCMPDQQS
IYAAQPMLNMHQMATANAFRGALSMTPIVSPQPTHLKPTIIVQQDSPMLMPLDTRFVSSDYYAF
PSTPPLSTSGSTISSPPSSGRSLHTPINDCFFSFEKVEGVKEGCESDVHSELLANADWSRSDSP
PLTPVFIHPPSLTASQSSDLLSAHSSCPSLSPSPSPVSSTFIAPPHSGLSVEPSGTDFCDPRQL
TVESSVDSSTELPPLPTLSCNEEEPKVVLGSATVTLPVHESLSPAYTSSTEDPLGSLPTFDSFT
DLDSEDEFVNNLVDFHPGGNPYFLGDKRQRLGSYLLEEDEFLSDRSFDDLDDHEAFAHSGLPSL
EPSELISVQGDVAEVSEEMRSKKRTTSRRTLKRTNSSDSSSESLATSGKRTQASANGRSGHSEA
TSSSAQQSTTPSRQNSTANASSSSEAPSAPVSVNRRGRKQSLTDDPSKTFVCTLCSRRFRRQEH
LKRHYRSLHTQDKPFECHECGKKFSRSDNLAQHARTHGGGSIVMGVIDTNASLQASYEEREPRL
LGAALYEAANAAANKSTTSDSSDGTISDTSSVEGRPIKKRRREDHA

[089] The amino acid sequence of the *Aspergillus fumigatus* Af93 Seb1 protein is shown, below, as SEQ ID NO: 7:

MDATYTMAQTPVQGQPSFAYYPTESQSRQQHFTSHPFEMQYYGQVSSYPQQQAQQQHSMPEQQP
VYAAQPMLNMHQMATTNAFRGALSMTPIASPQPTHLKPTIIVQQDSPALMPLDTRFVSNDFYGF
PSTPPLSTSGSTISSPPSSNGSLHTPINDCFFSFEKVEGVKEGCESDVHCELLANTDWSRSDSP
PLTPVFIQPQSLTASQSSDLLSAQIPCPSLSPSPSPDSATFISHPQSILSAEPSGSDFCDPRQL
TVESSVGAPAELPPLPTLSCNEEEPKVVLGSATVTLPVHEGLSPSFSSSSEDPLGSLPTFDSFS
DLDSEDEFANKLVDFHPIGNTYFQGDKRQRLGTYLLDEDEFLSERSLEDLDDQEAFAQSGLPSV
ESTDFLAVEGDATQSTEEMSSKKRVTSRRSLKKASTSESSSDSLAKKTQASATSRSGHSDTTST

VQQSTASSRQNSTANTSNSESPAAPVSVNRRGRKQSLTDDPSKTFVCSLCSRRFRRQEHLKRHY
RSLHTQDKPFECHECGKKFSRSDNLAQHARTHGGGSIVMGVIDTNSSNTQPAFDEPEPRALGLA
LYEAANAATSKSTTSESSDGTISDTSSVGGRPAKKRRRDDHV

[090] The amino acid sequence of the *Neosartorya fischeri* NRRL 181 Seb1 protein is shown, below, as SEQ ID NO: 8:

MDATYTMAQTPVQGQPSFAYYPTESQSRQQHFTSHPSEMQYYGQVPPYPQQQHSMPEQQPVYAA
QPMLNMHQMATTNAFRGALSMTPIASPQPTHLKPTIIVQQQDSPVLMPLDTRFVSNDFYGFPST
PPLSTSGSTISSPPSSNGSLHTPINDCFFSFEKVEGVKEGCESDVHCELLANTGWSRSDSPPLT
PVFIQPPSLTASQSSDLLSAHMSCPSLSPSPSPDSTTFISHPQSVLSAEPSGSDFCDPRQLTVE
SSVGAPAELPPLPTLSCNEEEPKVVLGSATVTLPVHEGLSPSFSSSSEDPLGSLPTFDSFSDLD
SEDEFANKLVDFHPIGNTYFLGDKRQRLGTYLLDEDEFLSERSLEDLDDQEAFAQSGLPSVESS
DFLAAEGDATQNTEEMSSKKRVTSRRSLKRASTSESSSDSLAKKTQASATSRSGHSETTSTVQQ
STASSRQNSTANTSSSGSPAAPVSVNRRGRKQSLTDDPSKTFVCSLCSRRFRRQEHLKRHYRSL
HTQDKPFECHECGKKFSRSDNLAQHARTHGGGSIVMGVIDTNGSNTQPAFDEPEPRALGLALYE
AANAATSKSTTSESSDGTISDTSSVGGRPAKKRRRDDHV

[091] In some embodiments of the present compositions and methods, the amino acid sequence of the Seb1 protein that is altered in production levels has a specified degree of overall amino acid sequence identity to the amino acid sequence of SEQ ID NOs: 4, 5, 6, 7, or 8, *e.g.*, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or even at least about 99% identity, to SEQ ID NOs: 4, 5, 6, 7, or 8. The polynucleotide sequences encoding SEQ ID NOs: 4, 5, 6, 7, or 8 can be found in the GenBank or JGI databases, as are known to one of skill in the art.

[092] In some embodiments of the present compositions and methods, a *seb1* gene is disrupted, wherein the *seb1* gene encodes a Seb1 protein that has a specified degree of overall amino acid sequence identity to the amino acid sequence of SEQ ID NOs: 4, 5, 6, 7, or 8, *e.g.*, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or even at least about 99% identity, to SEQ ID NOs: 4, 5, 6, 7, or 8.

[093] The skilled person will appreciate that genetic alterations that affect Seb1 production can be made in the same manner as genetic alterations that affect Mpg1 production, which are detailed,

herein. Alterations in the Seb1 protein resulting in alterations in viscosity are further described in Provisional Application No. 61/478,160, filed April 22, 2011, incorporated herein by reference.

V. Additive effect produced by altering Sfb3 production

[094] In some embodiments of the present compositions and methods, genetic alterations that affect Mpg1 production, or Mpg1 and Seb1 production, are combined with genetic alterations that affect Sfb3 production. The *Sfb3* gene (also known as *Lst1*) has previously been characterized in budding yeast (*i.e.*, *Saccharomyces cerevisiae*), where it encodes a protein associated with the COPII protein coat surrounding transport vesicles that carry proteins from the endoplasmic reticulum to the Golgi apparatus. *Sfb3*, as well as *Sfb2*, are homologs of *Sec24*, all of which genes are involved with packaging specific cargo proteins into the vesicles.

[095] While *Sec24* is an essential gene in yeast, *Sfb3* and *Sfb2* are not, although the deletion of *Sfb3* in yeast is known to affect the transport of a plasma membrane transport protein (Pma1p) and a glucanosyltransferase (Gas1p) that is involved in cell wall synthesis.

[096] Using BLAST to search the publicly available genome sequence of *Trichoderma reesei* using *S. cerevisiae* Sec24p, Sfb3p or Sfb2p amino acid sequences as query sequences reveals that *T. reesei* has a single gene that is most closely homologous to yeast Sec24 and a single gene that is most closely homologous to yeast Sfb3. No other homolog was identified suggesting that *T. reesei* does not have a gene equivalent to Sfb2. Moreover, homologs of the Sfb3 proteins were found in *e.g.*, *T. reesei* (SEQ ID NO: 9), *A. oryzae* (SEQ ID NO: 10), *A. niger* (SEQ ID NO: 11), *P. funiculosum* (SEQ ID NO: 12), *P. chrysogenum* (SEQ ID NO: 13), *N. Crassa* (SEQ ID NO: 14), and *F. oxysporum* (SEQ ID NO: 15):

[097] *Trichoderma reesei* Sfb3 amino acid sequence (SEQ ID NO: 9):

MDYTQYHALGHGEVLDPNDPNKTSAPAAPQFQPPSSPYVPPGSPYGAPPYHGGHQAPPMAMPPP
STPGYGPPQGQSFPGSPMPSQDAGLAAQFGGMSLGADAGGAAARKKKKDRHAYHSVEPTGSSQA
FNGLPPGTPAEQFLNVNNPQGIPALGGQFGSPLASPMGTPHMANPGQFPAPTSPFTPSAPVSPA
EFASRFGSPDAATSIGSAGPSQVSPDDMPSIPASRDAIQEHFFKNVYPTFERHVPPPATVSFVA
FDQGNASPKFTRLTLNNIPTTAEGLHATGLPLGMLIQPLAPLQAGEAEIPVLDFGDAGPPRCRR
CRAYINPFMMFRSGGNKFVCNLCSYPNETPPEYFCAVSPQGVRLDRDQRPELHRGTVEFVVPKE
YWTREPVGLRWLFVIDVTQESYNKGFMETFCEGILAALYGGNDEENDEDGEPKRRIPKGAKVGF
ITYDKDIHFYNINPHLDQAHMMIMPDLEDPFLPLGEGLFVDPYESKAIITSLLTRLPEMFSTIK
NPEPALLATLNAAVAALEATGGKVVCSCSTLPTWGPGRLFMRDDGNHPGGELDKKLYTTEHPAW
KKVSEKMASSGIGVDFFLAAPSGGYLDIATIGHVAATTGGETFYYPNFIAPRDGARLSMEITHA

ITRETGFQALMKVRCSTGLQVAAYHGNFVQHTFGADLEIGVIDADKALGVSFSHDGKLDPKLDA
HFQTALLYTTASGQRRVRCSNVIASVSDTSKESNTKELAIRQCLKFVDQDAVVGIFAKEASTKL
ATTSANLQDVRNWLTERTIDIMAYYKKHSANQFPPSQLVMPERLKEFCMYMLGMLKCRAFKGGI
ENSDRRVHELRMVRSMGPLELSLYLYPRMIALHNLQPEEGFADPETGHLKMPPSVRTSFSRVEP
GGVYLVDNGQQCLLWFHAQTSPNLITDLFGEGHDSLKGLDPYTSTLPVLETHLSAQVRNIIEFL
KSMRGSKGMTIQLARQGIDGAEYEFARMLVEDRNNEAKSYVDWLVHIHRGVQLELSGQRKKEGD
GEATAVMANFAGLRPAYW

[098] *Aspergillus oryzae* RIB40 Sfb3 amino acid sequence (GI: 83766074; SEQ ID NO: 10):

MADQSMYNTLGQGTSPAEDPSNPNRMAHQVPPQSQPAAGFPPGPYPPQPGAYYGNPPPNQYDAPAA
APPTQQLQSPPPRGLAPSPQLAYGTETQTHMGAPADPMAGLASQMSGLGIMGDSGARPGKKKHRHA
HHEIGGATASAPQQFAGMPQAGMQPSSQFLNTGLNQAPRPISPAAGVPPAGIVPQPGVPAPGSGSV
PTQGKIDPEQIPSIPQSRDIPTMYYFDHIYPTMERHLPPPAAVPFVAHDQGNSSPKHARLTLNNIP
TTSDFLSSTALPLGMVLQPLARLDPGEPEVPVLDFGEMGPPRCRRCRAYINPFMTFRSGGNKFVCN
MCTFPNDVAPEYFAPLDMSGARVDRLQRPELMIGTVEFMVPKEYWNKEPVGLQRLFLIDVSQESVN
RGFLKGVCKGITEALYGAPDASEEDAAARRVPEGSKIGIVTYDREVHFYNLSAQLDQAQMMVMTDL
EEPFVPLSEGLFVDPYESKDIITSLLHRIPKIFSHIKKPEPALLPALNAAMSALQATGGKIFASIC
SLPTWGPGALHMRDDPKVHGTDAERKLFTTDNQAWRTTAGKMAEHGIGVDMFVAAPGGTYVDVATI
GHVAEVSGGETFFYPNFHAPRDILKLSQEFAHAVTRETGYQAMMKVRCSNGLQVSAYHGNFIQHAL
GADLEIGSIDADKAIGVMFSYDGKLDPKLDAHFQAALLYTTAEGQRRVRCINVVAAVNEGGLETMK
FIDQDCVVSIMAKEAAAKTVDKSLKDIRASITEKTVDIFSGYRKVFSGSHPPGQLVLPENLKEFSM
YMLALIKSRAFKGGQEASDRRIHDMRMLRSIGATELALYLYPRVIPIHNMQPEDGFPNEQGQLQVP
PSLRASFSKIEEGGAYLVDNGQICLLWLHSRVSPNLLEDLLGPGQSSLQGLNPQTSSLPVLETHLN
AQVRNLLQYFSTMRGSKSVAIQLARQGLDGAEYEFARLLVEDRNNEAQSYVDWLVHIHRQINLELA
GHRKREDTSAEGSLTSLAGLRAPYW

[099] *Aspergillus niger* Sfb3 amino acid sequence (SEQ ID NO: 11)

MADPNMYHTYGQAPVPGENPSDPNQMAYQVPPQGYPAAGIPPGPSPPQPGAAYGVPAPNQQWPA
YGSPPPAQQPLQQPPSQFAHQADPQAAMGAPVDPGMAGLASQMSGLGIMGGEGGAARSSKKKHR
HAHHEIAGASASVAQPFAAAPQDPMQPTSQFLNTGLNQAPRPISPAASIPAPVNPAFGGGAGAV
PTQGKVDPEQIPSIPRSRDLPAQYYFNHVYPTMERHLPPPAAVPFVAHDQGNSSPKYARLTLNN
IPSTSDFLSSTGLPLGMVLQPLARLDGEQPIPVLDFGDAGPPRCRRCRAYINPFMSFRSGGNKF
VCNMCTFPNDVPPEYFAPLDPSGSRIDRMQRPELMMGTVEFLVPKDYWNKEPVGLQWLLLIDVS
QESVNKGFLKGVCKGIMEALYSEETENPEDEAPARRIPEGAKIGIVTYDKEVHFYNLSAQLDQA
QMMVMTDLEEPFVPLSEGLFVDPYESKDVITSLLQRIPSIFSHVKNPQPALLPALNAALSALRP

TGGKIVGTIASLPTWGPGALSLRDDPKVHGTDAERKLFTTEHAGWRETAGHLAEAGIGLDMFIA
APSGTYMDVATIGHIPEVTGGETFFYPNFHAPRDIRKLSKELAHAITRETGYQALMKVRCSNGL
QVSGYHGNFVQHTFGADLEIGAIDADKAIGVVFSYDGKLDPKLDAHFQAALLYTSANGQRRVRC
INTVAAVNEGGMETMKFVDQDAVVAMVAKDAASKTLDKSLKDIRAGVSEKTVDIFSGYRKIFSG
SHPPGQLVLPENLKEFSMYMLSLIKSRAIKGGQEASDRRIHDMRMLRSIGCTELSLYLYPRIIP
IHNMQPTDGFPNEQGQLQVPPSLRASFSKIEEGGAYLVDNGQQCLLWLHSHVSPNLLEDLFGEG
QTSLQGLSPQISTIPVLETHLNAQVRNLLQYFSTIRGSKAVTIQLARQGLDGAEYEFARMLVED
RNNEAQSSVDWLVHIHRQINLELAGHRKREDTAGEGGLTSLAGLRAPYW

[0100] *Penicillium funiculosum* Sfb3 amino acid sequence (SEQ ID NO: 12)

MADYSTYHSSGYAGAPGEDPNRQQPAVPAPYHSPNAPPGQAIQQPGITPYGAAQPPQFPGQPGV
GYGVAPVPSPPQALGGPDVGDLATRIGGLGIISDAGTRSHKKKHRHAYHDIGGPNAQGLNTFPS
QTNLQSQFLNTGLNQPEQQPAAPAAFPGAPVGQVPANVAPGAAPEVGGVGSVPTQGKIDPEQIP
SVPRSRDLPAQYYFNNVYPTMERHVPPPASIPFIAHDQGNSSPKVARLTLNNIPSSSDFLQSTG
LPLGMILQPLAKLDAGEQPVPVIDFGDIGPPRCRRCRTYINPFMTFRSGGNKFVCNMCTFPNDV
PPEYFAPVDPSGVRVDRLQRPELMLGTVEFTVPKEYWVKEPAGLHQLFLIDVSQESVNRGFLKG
VCDGIINALYGEEEPVEGAEPETRKVPEGSKIGIVTFDREIHFYNLLPRLDKAQMMVMTDLEEP
FVPLSEGLFVDPYESKDVITSLLEQLPSLFARVKSPESTLLPTIKAAISALQATGGKIICCLTS
LPTYGPGKLVMKDKSQAPDGENKLFAIDNPDYKAAATKLTEAGVGIDFFVAAPGGSFMDLTTIG
YTAAISGGECFFYPNFHSPRDSLKLAQEISHTVTRETGYQALMKVRCSNGLQVSAYYGNFLQHT
FGADLEIGTIDADKALGVLFSYDGKLDPKLDAHFQAALLYTAANGQRRVRCINIVAGVNEGGIE
TMKCIDQDAVVAIIAKEAASKAGDKTLKDIRASITEKTVDIFSGYRKNFSGSHPPGQLVLPENL
KEFSMYMLGLLKSRAFKGGSETADRRVHDLRMLRSIGCLELSLYLYPRIIPIHNMSAEDGFANE
QGQLQVPPALRASFSRVEEGGAYLIDNGQGILLWIHSFVSPNLLEDLFGPGITSLQALDPNTSS
LPVLETHLNAQVRNLLQYLSTVRGSKAVTIQLARQGIDGAEYEFARSLVEDRNNEAQSYVDWLV
HIHRQINLELAGHRKKEDSATSSGEGALSSLAGIRAPYW

[0101] *Penicillium chrysogenum* Sfb3 amino acid sequence (SEQ ID NO: 13)

MADSSMYNTMGQGSSEDPSNPQYMAQVPPQQYPAGYPPTAAPLQPGAPYANPAPNQWPAYGSPQ
QPGMASPGIAYNAPQQPMGAAVDPGMAGLASQMGGLDIAADAGARTHRKKHRHAHHDIGGGAAP
PAQGFNTGMDQGGLQQPQPQQQSQFLNTGLNQHADRPVSPAVGLVSGQSVAAIPGIQSGAGSVP
TSGRIDPEHIPSIPRSRDLPAQYYFNHVYPTMDQHLPPPAAIPFVAQDQGNSSPKYARLTLNNI
PSASDFLTSTGLPLGMILQPLAPLDPGEQPIPVLDFGDVGPPRCRRCRTYINPFMSFRSGGSKF
VCNMCTFPNDTPPEYFAPLDPSGARVDRMQRPELLMGTVEFTVPKEYWNKEPVGLQTLFLIDVS
RESVHRGFLKGVCAGIKDALYGDDDKASEGTEGDGSSRKLPVGAKVGIVTYDKEVHFYNLAAAL

DQAQMMVMTDLDEPFVPLSEGLFVDPYESKSVITSLLSRIPKIFSSIKNPESALLPTLNSALSA
LQATGGKIVCAVASLPTCGPGHLAIREDPKVHGTDAERKLFTTENPAWKKTASKLAEAGVGLDL
FMAAPGGTYLDVATIGHVSSLTGGETFFYPNFHAPRDLLKLRKEIAHAVTRETGYQTLMKVRCS
NGLQVSAYHGNFVQHTLGADLEIAGVDADKAVGVLFSYDGKLDPKLDAHFQAALLYTSADGQRR
VRCINVVAAVNEGGLETMKFVDQDAVVSVIAKEAASKTLDKNLKDIRASISEKTVDIFSGYRKI
FSGSHPPGQLVLPENLKEFSMYMLSLVKSRAFKAGPESSDRRIHDMRLIRSMGCTEMALYLYPR
IIPVHNMQPEDGFANEHGQLQIPPTMRASYSRIEDGGVYIVDNGQAILLWIHAQVSPNLLEDLF
GPGHNSLQGLNPNTSSLPVLETHLNAQVRNLLQYLSTVRGSKSVTIQLARQGLDGAEYEFARLL
LEDRNNEAQSYVDWLVHIHRQINLELAGHRKKEEGGEGALASLSAMRTPYW

[0102] *Neurospora crassa* Sfb3 amino acid sequence (SEQ ID NO: 14)

MADYTMYHALGQGETLDPNDPNRTTQPAPPQFQPPVAPNPYHPGAEYNAPGQQQQQQQQYGQQY
GQQYGQQYGQQQYGQEYGHQQQQQQQQQYGAPSPYGAPPAHSPVSPMDDVGLAAQMGGMSLGAG
AGAADHHGRKKKKDRHAFHTVEAPAGSSQPFNGMPPAGIPATQFLNADPSLAGRIPGPGHGQFP
MPASPAFGPVPTSAADFAARDATQGVGSGVFAAGGPQGGKPSPDDTPSVPLSRDAVQPYFHTNV
YPTFERLVPPPAVTSFVALDQGNSSPKFARLTMTNLPASAEGLKSTGLPLGLLLQPLAETQPGE
LPIPVLDFGEQGPPRCHRCRAYMNPFMMFKAGGNKFVCNLCTYANDTPPEYFCALSPQGVRVDR
DQRPELTRGTVEFVVPKEYWTKEPVGMRYLFVIDVTQESYNKGFLESFCEGILSALYGGSEEGE
DQDETGEPKRKIPAGAKVGFVTFDQEIHFYNVSPALEQAQMIVMPDIEDPFLPLSDGLFVDPYE
SKAVISSLLTRLPQMFSNIKNPEPALLSALNSAVAALEKTGGKVFCSLAALPTWGPGRLFMRDD
GKHPGGEPDKKLFTTEHPGWRKLAEKMVSLGVGADFFMASPSGGYLDIATIGHVSSTTGGETFF
YPNFVVQRDSTKLSLEIHHAVRRETGYAALMKVRCSNGLQVNAYHGNFIQHTFGADLEIGVIDA
DKALAVTFGYDGKLDSKLDAHFQAALLYTTASGQRRVRCINVIAGVSDLARDCMKYIDQDAIVS
ILAKEASTKLSTTSANLKEVRSSLTEKTIDILALYRKNHLAVPHPPQQLVMPERLKEFTMYVLG
MLKCRAFKGGNETTDRRVHDMRLIRSMGARELSLYLYPRIIPLHSLQPEDGYPDATTGHLRMPS
TMRASFARVEPGGVYLVDNGQVCLLWMHAQTAPALIQDLFGEDKTTLQSLDPYTSTIPVLETHL
NAQTRNIIEYMRTVRGSKGLTIQLARQGIDGAEFEFARMLVEDRNNEAQSYVDWLVHVHKGVQL
ELAGQRKREDGESHSALGSFTGLRPAYW

[0103] *Fusarium oxysporum* Sfb3 amino acid sequence (SEQ ID NO: 15)

MADYAQYHALGQGEVIDPNDPNRTSQPSAQQFQPPIAPSPYQQQASPYGAPQYLGGQQAPPPMT
GSPAPAPGYGYAPPQAQAPPGQAPPSQDATLAAQLGGMNLGDGHGTARRKKKDRHAYHTVEPTG
SSQAFNGMPPQGTSATQFLDSVPGGPGFGGQFGSPQGTPQMQSQSQFSAPVNPAFGPGPVAGTP
GVGEGLGTASVSTSGPKGVSPDDMPSVPASRDAIQQYYLKNVYPTFERHVPPPSTVSFVAYDQG
NSSPKYTRLTLNNIPTTQDALQATGLSLGLLLQPLAPLQAGEAEIPVLDFGEAGPPRCRRCRAY
MNPFMMFRSGGNKFVCNLCAYPNDTPPEYFSATNPQGVRVDRDTRPELHRGTVEFVVPKEYWTR

EPVGLRWLFLIDVTQESYNKGYVEAFCEGIRVALYGGEDQELDENGEPKRRIPEGAKVGFVTYD
KDIHFYNVNPALDQAQMMIMPDLEDPFVPLSEGLFVDPYESKDVITSLLTRLPDMFSTIKNPEP
ALLAALNSALAALEATGGKVVASCSALPTWGPGRLFMRDNGNHPGGEIDKKLYTTEHPAWKKVA
EKMAASGVGADFFLAAPSGGYLDIATIGHVSSTTGGETFYYPNFIAARDSRKLSLEISHAVTRE
TGFQALMKVRCSNGLQVSGYHGNFIQHTFGADLEIGVIDADKAMGVSFSYDGKLDPKLDAHFQS
ALLYTTASGERRVRCSNVIASVTETSKESGAREQGIRECLKFVDQDAVIGMLAKEASTKLATTS
SNLKDIRHWLSEKAIDVLACYRKHAAQQHPPGQLVMPERLKEYCMYLLGLLKCRALKGGVENSD
RRVHEMRMLRSMGALELSLYLYPRMIPIHNLAPEEGFADPETGHLKMPPAIRTSFSRVEPGGVY
LVDNGQQCLLWFHSQTSPNLISDLFGEDKDSLKSLDPYTSALPLLETHLNAQVRNIIEFLRTMR
GSKGLTIQLARQGIDGAEFDFARMLVEDRNNEAQSYVDWLVHIHKGVQLELSGQRKKEGEEHTA
ASLSNFAGLRPAYW

[0104] In some embodiments of the present compositions and methods, the amino acid sequence of the Sfb3 protein that is altered in production levels has a specified degree of overall amino acid sequence identity to the amino acid sequence of SEQ ID NOs: 9. 10, 11, 12, 13, 14, or 15, *e.g.*, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or even at least about 99% identity, to SEQ ID NOs: 9. 10, 11, 12, 13, 14, or 15. The nucleotide sequences encoding each amino acid sequence can be identified from a BLAST search for each corresponding protein as is know to one skilled in the art.

[0105] In some embodiments of the present compositions and methods, a *sfb3* gene is disrupted, wherein the *sfb3* gene encodes a Sfb3 protein that has a specified degree of overall amino acid sequence identity to the amino acid sequence of SEQ ID NOs: 9. 10, 11, 12, 13, 14, or 15, *e.g.*, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or even at least about 99% identity, to SEQ ID NOs: 9. 10, 11, 12, 13, 14, or 15.

[0106] An alignment of the amino acid sequences of the Sfb3 proteins from approximately 40 Pezizomycotina species revealed a specific amino acid sequence, *i.e.*, IQLARQGXDGXEXXXARXLXEDRNXEAXSXVDWL (SEQ ID NO: 16, where X is any amino acid residue), which is close to the C-terminus of the Sfb3 proteins, and not found in Sec24 proteins. This consensus sequence can be used to identify Sfb3 proteins and variants thereof in other members of the Pezizomycotina.

Sec24 proteins. This consensus sequence can be used to identify Sfb3 proteins and variants thereof in other members of the Pezizomycotina.

[0107] The skilled person will appreciate that genetic alterations that affect Sfb3 production can be made in the same manner as genetic alterations that affect Mpg1 and/or Seb1 production, which are detailed, herein. Alterations in the Sfb3 protein resulting in alterations in viscosity are further described in PCT Publication No. WO 2012/027580 A1, published 1, March 2012, filed as International Application No. PCT/US2011/049164, filed 25, August 2011.

VI. Utility

[0108] The use of reduced viscosity strains of filamentous fungi is known to improve the distribution of oxygen and nutrients in a submerged culture, reduce the amount of energy required to agitate a submerged culture, and increase the cell mass present in the culture, leading to increased protein production. Moreover, the present variant strains of filamentous fungus offer significant advantages over previously-described reduced viscosity strains.

[0109] First, the present variant strains can have a fully defined genome, making them well-suited for subsequent genetic manipulation, complementation, mating, and the like. Second, the present strains are not adversely affected in protein production, for example, by the manipulation(s) that resulted in the attendant viscosity alteration. Third, reduced viscosity strains can be produced from essentially any parental strain, including parental strains that already produce a protein intended for high level expression (*i.e.*, a protein of interest), already encode a selectable marker, or already include other features that are desirable in a production host. Thus, the present strain and methods eliminate the need to transfer a gene encoding a protein of interest into a preexisting reduced viscosity production strain.

[0110] The present strains and methods find use in the production of commercially important protein in submerged cultures of filamentous fungi. Commercially important proteins include, for example, cellulases, xylanases, pectinases, lyases, proteases, kinases, amylases, pullulanases, lipases, esterases, perhydrolases, transferases, laccases, catalases, oxidases, reductases, chlorophyllases, hydrophobin, chymosin, carbonic anhydrase, hymidylate synthase, dihydrofolate reductase, tyrosine kinases, multi-drug resistance proteins (e.g., ABC P-gp proteins), CAD (carbamyl-P synthase, aspartate transcarbamylase, dihydroorotase), topoisomerases, ribonucleotide reductase, and antibodies and other enzymes and non-enzyme proteins capable of being expressed in filamentous fungi. Such proteins can be suitable for industrial, pharmaceutical, animal health and food and beverage use.

can be used alone or in combination with the subject matter of any other numbered paragraph, as indicated.

1. In one aspect, a variant strain of filamentous fungus derived from a parental strain is provided, the variant strain comprising a genetic alteration that causes cells of the variant strain to produce an altered amount of functional Mpg1 protein compared to cells of the parental strain, wherein the cells of the variant strain produce during aerobic fermentation in submerged culture a cell broth that (i) requires an altered amount of agitation to maintain a preselected dissolved oxygen content compared to the cells of the parental strain, and/or (ii) maintains an altered dissolved oxygen content at a preselected amount of agitation, compared to the cells of the parental strain.

2. In some embodiments of the variant strain of paragraph 1, the altered amount of functional Mpg1 protein is a reduced amount, and the variant strain produces during aerobic fermentation in submerged culture a cell broth that (i) requires reduced agitation to maintain a preselected dissolved oxygen content compared to the cells of the parental strain, and/or (ii) maintains an increased dissolved oxygen content at a preselected amount of agitation, compared to the cells of the parental strain.

3. In some embodiments of the variant strain of paragraphs 1 or 2, the genetic alteration comprises a disruption of the *mpg1* gene present in the parental strain.

4. In some embodiments of the variant strain of paragraph 3, disruption of the *mpg1* gene is the result of deletion of all or part of the *mpg1* gene.

5. In some embodiments of the variant strain of paragraph 3, disruption of the *mpg1* gene is the result of deletion of a portion of genomic DNA comprising the *mpg1* gene.

6. In some embodiments of the variant strain of paragraph 3, disruption of the *mpg1* gene is the result of mutagenesis of the *mpg1* gene.

7. In some embodiments of the variant strain of any of paragraphs 3-6, disruption of the *mpg1* gene is performed using site-specific recombination.

8. In some embodiments of the variant strain of any of paragraphs 3-7, disruption of the *mpg1* gene is performed in combination with introducing a selectable marker at the genetic locus of the *mpg1* gene.

9. In some embodiments of the variant strain of any of paragraphs 1-8, the variant strain does not produce functional Mpg1 protein.

10. In some embodiments of the variant strain of any of paragraphs 1-8, the variant strain does not produce Mpg1 protein.

11. In some embodiments of the variant strain of any of paragraphs 1-10, the variant strain further comprises a gene encoding a protein of interest.

12. In some embodiments of the variant strain of any of paragraphs 1-11, further comprises a disruption of the *sfb3* gene.

13. In some embodiments of the variant strain of any of paragraphs 1-12, further comprises a disruption of at least one gene selected from the group consisting of the *sfb3* gene, the *seb1* gene, the *gas1* gene, the *crz1* gene, and the *tps2*.

14. In some embodiments of the variant strain of any of paragraphs 1-13, the variant strain produces substantially the same amount of, or more, protein per unit amount of biomass as the parental strain.

15. In some embodiments of the variant strain of any of paragraphs 1-14, the filamentous fungus is a Pezizomycotina species.

16. In some embodiments of the variant strain of any of paragraphs 1-15, the filamentous fungus is a *Trichoderma* spp.

17. In some embodiments of the variant strain of any of paragraphs 1-16, the filamentous fungus is *Trichoderma reesei.*

18. In another aspect, a method for producing a variant strain of filamentous fungus cells is provided, comprising: introducing a genetic alteration into a parental strain of filamentous fungal cell, which genetic alteration alters the production of functional Mpg1 protein compared to the cells of the parental strain, thereby producing a variant filamentous fungal cell that produces during aerobic fermentation in submerged culture a cell broth that (i) requires an altered amount of agitation to maintain a preselected dissolved oxygen content, compared to the cells of the parental strain, and/or (ii) maintains an altered dissolved oxygen content at a preselected amount of agitation, compared to the cells of the parental strain.

19. In some embodiments of the method of paragraph 18, the genetic alteration reduces or prevents the production of functional Mpg1 protein, thereby producing a variant filamentous fungal cell that produces during aerobic fermentation in submerged culture a cell broth that (i) requires reduced agitation to maintain a preselected dissolved oxygen content, compared to the cells of the parental strain, and/or (ii) maintains an increased dissolved oxygen content at a preselected amount of agitation, compared to the cells of the parental strain.

20. In some embodiments of the method of paragraph 18 or 19, the genetic alteration comprises disrupting the *mpg1* gene in a parental filamentous fungal cell using genetic manipulation.

21. In some embodiments of the method of any of paragraphs 18-20, the genetic alteration comprises deleting the *mpg1* gene in a parental filamentous fungal cell using genetic manipulation.

22. In some embodiments of the method of any of paragraphs 18-21, the genetic alteration is performed using site-specific genetic recombination.

23. In some embodiments of the method of any of paragraphs 18-22, disruption of the *mpg1* gene is performed in combination with introducing a selectable marker at the genetic locus of the *mpg1* gene.

24. In some embodiments of the method of any of paragraphs 18-23, disruption of the *mpg1* gene is performed in combination with disrupting the *sfb3* gene.

25. In some embodiments of the method of any of paragraphs 18-24, disruption of the *mpg1* gene is performed in combination with disruption of at least one gene selected from the group consisting of the *sfb3* gene, the *seb1* gene, the *gas1* gene, the *crz1* gene, and the *tps2* gene.

26. In some embodiments of the method of any of paragraphs 18-25, the variant strain produces substantially the same amount of, or more, protein per unit amount of biomass as the parental strain.

27. In some embodiments of the method of any of paragraphs 18-26, the filamentous fungus is a Pezizomycotina species.

28. In some embodiments of the method of any of paragraphs 18-27, the filamentous fungus is a *Trichoderma* spp.

29. In some embodiments of the method of any of paragraphs 18-28, the filamentous fungus is *Trichoderma reesei*.

30. In some embodiments of the method of any of paragraphs 18-29, the parental strain further comprises a gene encoding a protein of interest.

31. In some embodiments of the method of paragraph 30, the gene encoding the protein of interest is present in the parental strain prior to introducing the genetic alteration that reduces or prevents the production of functional Mpg1 protein.

32. In another aspect, a protein of interest produced by the variant strain of paragraph 11 is provided.

33. In another aspect, a variant strain of filamentous fungus produced by the method of any of paragraphs 18-31 is provided.

34. In another aspect, a variant strain of filamentous fungus derived from a parental strain is provided, the variant strain comprising:

(a) a genetic alteration that results in (i) a requirement for reduced agitation in submerged culture to maintain a preselected dissolved oxygen content, compared to the cells of the parental strain, and/or (ii) maintenance of an increased dissolved oxygen content in submerged culture at a preselected amount of agitation, compared to the cells of the parental strain, and

(b) a gene encoding a protein of interest,

wherein the gene encoding the protein of interest is present in the variant strain prior to the genetic alteration in (a).

35. In some embodiments of the variant strain of paragraph 34, the genetic alteration comprises a disruption of the *mpg1* gene present in the parental strain.

36. In some embodiments of the variant strain of paragraph 35, disruption of the *mpg1* gene is performed in combination with introducing a selectable marker at the genetic locus of the *mpg1* gene.

37. In some embodiments of the variant strain of paragraph 35 or 36, disruption of the *mpg1* gene is performed in combination with disrupting at least one gene selected from the group consisting of the *sfb3* gene, the *seb1* gene, the *gas1* gene, the *crz1* gene, and the *tps2* gene.

38. In some embodiments of the variant strain of any of paragraphs 35-37, disruption of the *mpg1* gene is performed in combination with disrupting the *seb1* gene.

[0112] These and other aspects and embodiments of the present strains and methods will be apparent to the skilled person in view of the present description. The following examples are intended to further illustrate, but not limit, the strains and methods.

EXAMPLES

Example 1. Identification of the *mpg1* gene as responsible for morphological changes in filamentous fungus

A. Overview

[0113] Filamentous fungi disruption libraries were prepared by transforming an exemplary filamentous fungus, *i.e.*, *Trichoderma reesei*, with a nucleic acid containing the *pyr2* gene and the *T. reesei* histone H1 promoter, using *Agrobacterium tumefaciens*-mediated transformation. In this manner, the *pyr2* gene served as both a selectable marker and a gene tag. The histone H1 promoter also served as a gene tag and as a promoter to upregulate genes if inserted before the start codon of a gene. The particular *A. tumefaciens* strain used was EHA 105, which is considered to be a hypervirulent (Hood *et al.*, 1993). However, other *A. tumefaciens* strains, *e.g.*, A136 and EHA 101, produce similar transformation frequencies in *T. reesei*. *A. rhizogenes*

strains, *e.g.*, ATCC 43057, can also be used. The particular disruption library contained about 50,000 transformants.

B. *Trichoderma reesei* MAGI strain

[0114] The *T. reesei* Morph 1.1 (*i.e.*, "Morph") mutant is deleted for four major cellulases genes (*i.e.*, *cbhI, cbhII, eglI and eglII*), which makes it useful for expressing other proteins in the absence of cellulase background activity. The MAGI strain was generated by targeting the insertion of a reporter cassette to the orotidine 5'-monophosphate pyrophosphorylase (*pyr2)* locus of *Trichoderma reesei* Morph 1.1. This reporter cassette contains a codon optimized green fluorescent protein (GFP) from *Ptilosarcus species* and an alpha-amylase under the control of the *T. reesei* cellobiohydrolase I (*cbhI*) promoter and transcriptional terminator sequences. A hygromycin B phosphotransferase gene is also integrated with the reporters at the *pyr2* locus. Coincident with integration of the reporter cassette, a 3′ portion of the *pyr2* gene is deleted making the strains uridine auxotrophs.

C. Preparation of DNA

[0115] The vector used for disruption was pRATT 236 based on the PZP 100 vector, which includes the left and right T-DNA border regions, a pBR322 *bom* site for mobilization from *E. coli* to *Agrobacterium*, ColE1 and pVS1 plasmid origins for replication in *E. coli* and *Agrobacterium*, respectively, and a bacterial marker for conferring chloramphenicol resistance (Hajdukiewiez, O. *et al.*, 1994). A representation of the vector is shown in Figure 1.

[0116] A disruption cassette containing the *pyr2* gene of *Trichoderma atroviride* followed by the *his1* promoter oriented to transcribe outward into the insertion site was prepared by standard molecular biology techniques and ligated to generate the pRATT 236 vector. The resulting vector was propagated in *E. coli* TOP10 cells (Invitrogen, Carlsbad, CA, USA). LA agar plates (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl, 10 g/L agar) with 25 ppm chloramphenicol were used to select for *E. coli* transformants. *E. coli* containing the vector were grown in LB medium (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl) plus 25 ppm chloramphenicol. Vector DNA was isolated using standard methods.

D. Transformation of *Agrobacterium* cells

[0117] Competent *Agrobacterium* cells were made as follows. Briefly, *Agrobacterium* cells were revived from cryopreservation by growing on LA medium at 28°C for about three days. Colonies were then selected and grown in LB medium containing 0.1 % glucose in 50 ml volumes in 250 ml dented bottom flasks at 28°C until growth was apparent. Alternatively, colonies were started in a 5 ml culture tube and transferred to a 250 ml flask when growth was apparent. About 10% of the volume of the 250 ml flask was then transferred into a fresh flask

with the same medium, which was grown to an OD (600 nm; OD_{600}) of about 0.4-0.8 (about 5-6 hours of growth). The cells were recovered by centrifugation in a cold centrifuge at 10,000 rpm for 10 minutes, and then washed three times in cold 1 M HEPES, pH 7.0. Next, the cells were washed once in cold 1 mM HEPES with 10 % glycerol, and aliquots were frozen at -70 °C. Cell viability was determined (typically about $1x10^9$ CFU/ml after freezing).

[0118] The vector DNA was used to transform *Agrobacterium* cells by electroporation. Competent *Agrobacterium* cells were thawed on ice and about 40 µl of the cells were mixed with about 1 µg of DNA in a 0.2 cm electroporation cell (on ice). The cells were electroporated at 2.5 volts (200 Ohms, at 25 µF) with a Buchler 3-150 electroporator. SOC medium (Invitrogen) was added to the electroporation cell immediately after electroporation. Alternatively, the *Agrobacterium* cells can be transformed by electroporation using the ligation mixture, thereby eliminating the need to propagate the vector DNA in *E. coli*. In the alternative method, about 1 µl of the ligation mixture is used for transformation. After the addition of SOC to the electroporation mixture, dilutions of the mixture were plated onto LA medium plus 250 ppm chloramphenicol culture plates and incubated at 28 °C for four days. 1 x 10^7 CFU/ml of *Agrobacterium* transformants were obtained and about 90-100 % contained the vector DNA, as determined by PCR analysis. As little at 25 ppm chloramphenicol can be used to obtain colonies in a shorter time frame but a larger number of colonies must to be screened to identify *bonafide* transformants.

E. *Agrobacterium*–mediated transformation of *T. reesei*

[0119] 25 ml of minimal medium (2.05 g/L K_2HPO_4, 1.45 g/L KH_2PO_4, 0.15 g/L NaCl, 0.5 g/L $MgSO_4 \cdot 7 \cdot H_2O$, 0.1 g/L $CaCl_2 \cdot 6 \cdot H_2O$, 0.0025 g/L, $FeSO_4 \cdot 7 \cdot H_2O$, 0.5 g/L $(NH_4)_2SO_4$, and 2 g/L glucose, with 25 ppm chloramphenicol added after sterilization) in a 250 ml flask was inoculated with either a frozen stock of vector-transformed *Agrobacterium* or directly from a fresh LA plate. The minimal medium culture was then incubated at 28 °C with shaking until cloudy (overnight to several days). 10 ml of the culture was transferred to 50 ml of induction medium (2.05 g/L K_2HPO_4, 1.45 g/L KH_2PO_4, 0.15 g/L NaCl, 0.5 g/L $MgSO_4 \cdot 7 \cdot H_2O$, 0.1 g/L $CaCl_2 \cdot 6 \cdot H_2O$, 0.0025 g/L, $FeSO_4 \cdot 7 \cdot H_2O$, 0.5 g/L $(NH_4)_2SO_4$, 1.8 g/L glucose, 5 g/L glycerol, prepared in 40 mM MES, pH 5.3, with 200 µL of 1 M acetosyringone added after sterilization) in 250 ml flasks. The staring OD_{600} was about 0.1, and the vector-transformed *Agrobacterium* cells were grown to an OD_{600} of about 0.4–0.8.

[0120] A fresh culture of *T. reesei* MAGI cells was prepared by resuspending spores in 10 ml of sterile water. Transformation of the *T. reesei* MAGI cells was performed as follows: About 100 µl of *Agrobacterium* whole broth (OD_{600} = 0.4–0.8) was mixed with 100 µl of fungal spores (10^7

sfu/ml) in a tube (other ratios of *Agrobacterium* cells to fungal spores will also produce satisfactory results). About 0.1-1.0 ml of this mix was plated onto induction agar plates (induction medium with 15 g/L agar and 0.25 mg/mL uridine) with embedded nitrocellulose filters. The plates were incubated at about 18-28℃ for about 24-48 hours to allow the growth of the *T. reesei* cells. Next, the nitrocellulose filters were transferred to Vogel's medium (Vogel, *Microbiol. Genet. Bull.* 13:42-43, 1956) supplemented with 250 ppm carbenicillin to kill/inhibit *Agrobacterium* growth. The cultures were then incubated at 28℃ until growth of filamentous fungi (representing transformants of the disruption library) on the filters was evident.

F. Screening for morphology mutants

[0121] Transformants in the disruption library were screened for alterations in morphology in solid and liquid culture using light microscopy. Following transformation, individual transformants were picked from the nitrocellulose filters using a colony picker and transferred to 96-well microtiter plates containing potato dextrose agar (CP-700, Norgren Systems LLC, Fairlea, WV, USA). Alternatively, spores from transformants were combined, germinated, and single spores were added to microtiter wells using a cell sorter. Spores were collected by suspending spores from a potato dextrose transformation plate in 20 ml sterile distilled water using a cell spreader. Spores were inoculated into a 250 mL flask containing 50 ml of a minimal medium and incubated at 28°C with agitation for 24 h until germlings were obtained. Using high speed sorting (MoFlo sorter, Cytomation, Fort Collins, CO, USA) at an event rate of 15,000 event per second, 60 psi with a 70 µm nozzle), individual germlings were separated into microtiter plate wells containing potato dextrose agar (Difco, Detroit, MI, USA). The microtiter plates containing the transformants obtained by either method described above, were incubated for 7 days at 28°C. The individual germinates spores were replicate plated into 384 well black sensoplates with glass bottoms (Greiner Bio-one, Germany) containing YEG (5 g yeast extract, 20 g glucose per 1 L water) and incubated at 20°C, for 24 h. The morphology of individual transformants was examined microscopically.

[0122] Alternately, slow growing transformants were isolated directly from the transformation plates and re-plated on potato dextrose agar (Difco). Transformants showing colonial growth on the potato dextrose plates were grown in YEG medium in shake flasks at 28 °C, 150 rpm, for 24 h and the morphology of the transformants was examined microscopically.

G. Isolation and characterization of *T. reesei* MAGI 10-8g

[0123] Mutant MAGI 10-8g obtained from the above procedure was observed to have altered morphology in liquid culture having shorter filaments than the MAGI parent. In liquid medium, cultures containing the MAGI 10-8g mutant also showed a higher level of dissolved oxygen during growth compared to cultures containing the MAGI parent (Table 1).

[0124] Strains MAGI and MAGI 10-8g were grown under similar conditions in submerged (liquid) culture, and their growth phenotypes were compared. Briefly, spores of each strain were added separately to 500-mL of minimal medium in a 3-L flask with both side and bottom baffles. After autoclaving for 30 minutes, sterile 60% glucose was added to a final concentration of 27.5 g/L. Since the MAGI strain is Δ*pyr2* it was supplemented with 2 mg/ mL uridine. The culture was grown for 48 hrs at 34°C in a shaking incubator.

[0125] After 48 hrs, the contents of each flask were added separately to 14-L fermentors containing 9.5 L of medium containing 4.7 g/L KH_2PO_4, 1.0 g/L $MgSO_4 \cdot 7 \cdot H_2O$, 4.3 g/L $(NH_4)_2SO_4$ and 2.5 mL/L of the same trace element solution. These components were heat sterilized together at 121°C for 30 minutes. A solution of 60% glucose and 0.48% $CaCl_2 \cdot 2 \cdot H_2O$ was separately autoclaved, cooled, and added to the fermentor to a final concentration of 75 g/L glucose and 0.6 g/L $CaCl_2 \cdot 2 \cdot H_2O$. The medium was adjusted to pH 3.5 with 28% NH_3 and the temperature was maintained at 34°C for the entire growth period.

[0126] A dissolved oxygen (DO) probe was calibrated to 100% when there was no added pressure in the headspace (*i.e.*, 0 bar gauge, 1 bar absolute). The pressure in the headspace was then set to 0.7 bar (gauge), after which the oxygen probe read 170% before the seed culture was added. The fermentor contained two, four-blade turbines that provided mixing via a variable speed motor that was initially set at 500 rpm.

[0127] As the cultures grew, DO content levels dropped, at least partly as a consequence of the increased viscosity of the broth due to the proliferation of filamentous fungus hyphae. When DO content levels fell below 40%, the agitation rate was increased to maintain the dissolved oxygen at 40%. Upon reaching 750 rpm agitation, DO content level would be allowed to drop below 40%. If the DO content did not fall below 40%, then it was unnecessary to increase the agitation rate during the fermentation run, and the initial agitation rate was higher than necessary. When the glucose was completely consumed, the amount of biomass produced in each fermentor was measured, and found to be substantially the same for all both strains.

[0128] The DO content level in each fermentor at a given level of agitation, and the amount of agitation required to maintain a given DO content level are indirect measures of the viscosity of

the different broths, due to the different strain growth phenotypes. Although it would be ideal to vary only one variable (*i.e.*, DO or agitation) and measure the other, it is desirable to prevent the DO from falling below 40% to in production of sufficient biomass in each fermentor, thereby permitting a more meaningful comparison between the growth of the different strains.

[0129] Generally, where it is necessary to increase the agitation rate to maintain a target DO level, the amount of agitation can be estimated by the amount of power supplied to the motor driving the fermentor turbine, which provides a metric that correlates with the viscosity of the broth. In particular, the extra power required to agitate the suspended culture is proportional to the agitation rate raised to the 3rd power.

[0130] The nucleic acid sequence of the *mpg1* gene was obtained from the JGI data base: Protein ID: 122551, Name: estExt_fgenesh5_pg.C_130115, available at: http://genome.jgi-psf.org/cgi-bin/dispGeneModel?db=Trire2&id=122551, (The Genome Portal of the Department of Energy Joint Genome Institute I. V. Grigoriev, H. Nordberg, I. Shabalov, A. Aerts, M. Cantor, D. Goodstein, A. Kuo, S. Minovitsky, R. Nikitin, R. A. Ohm, R. Otillar, A. Poliakov, I. Ratnere, R. Riley, T. Smirnova, D. Rokhsar, and I. Dubchak. Nucleic Acids Res 2011 0: gkr947v1-gkr947) as disclosed below. The untranslated region is italicized and flanked 5' and 3' by upstream or downstream sequence, coding regions are in bold and introns are in lower case (SEQ ID NO: 38):

GGCAAGGCGTACGCATGAGCGGAGCGGCAGTAGGTACTTGCGCCTCCGTGCTCATCTGCTGCCC
GCAGCGCGTACCGGCGTCGTGACATCTGGACACCTCGTTCGTCCCTACTTTAGATCCATCCAGC
CCGAACCTCATTTTCCTCTCTCCTTTTCCCTTCCATCCTCCCGCAACCACCGCGTCTTTTCTTC
CCTCCCGA*GCCGACACTCGAGTCTCTGCCCTGCGAGCATTGCACCGTCGCTCGTTCTTCTCTAC*
GCTCACTATCCAACATACTAGTTTATTCTTTTTCCCTTCTTCTACCATCTTCTGCCTCTTTACT
TACGAAATCAAACCCCCCCCTTTAAAACATCCACGAATCTCCTTTGCACTTCAGCTTCGTCGCA
*TACATTCACC***ATGAAGG**gtaggtgacgcgccggttccccaatctgcccatcattggcttcactc
cagctccaatggcaagatctcgctgacaatctctctcccctgcgcag**GACTTATTCTTGTCGGC**
GGCTTTGGCACTCGCCTTCGCCCTCTCgtacgtccacgccagcaccaccagcagcgatccgacc
tgcatcccactaccgcattgacgcggatggggtggcatggaggggaaaaccaccataagcgca
gcctctcacacccgcgaacctccactgaccattgtgcgacgccaatctag**ACCCTGACGCTCCC**
CAAGCCTCTGGTTGAGTTCTGCAACAAGCCCATGATTGTGCACCAGATCGAGGCTCTCGTCGCC
GCTGGCGTGACCGACATTGTCCTCGCCGTCAACTACCGCCCAGAAATCATGGAAAAGTTCCTGG
CCGAGgtgagtcgtgcacatcacaccctatgacccctcactacaaacccttgcctattcgcctg
cccattcgctgtaccaagcttttcgccccccccccccccccctcccctcccctcctactcagc
atatctcccccccaccaatgacaatggacgcaaaggctgattgcgtacgctcgaccgtttag**TA**

CGAGGAGAAATACAACATCAACATTGAGTTCTCCGTCGAGTCGGAGCCCCTCGACACCGCCGGC
CCCCTCAAGCTTGCTGAGCGCATCCTCGGCAAGGATGACTCGCCCTTCTTCGTCCTCAACTCCG
ACGTCATCTGCGACTATCCCTTCAAGGAGCTCCTCGAGTTCCACAAGGCCCACGGCGATGAGGG
CACCATTGTCGTCACCAAGGTCGAGGAGCCGTCCAAGTACGGTGTCGTCGTCCACAAGCCCAAC
CACCCCTCGCGCATCGACCGCTTCGTCGAGAAGCCCGTCGAGTTCGTCGGCAACCGCATCAACG
CCGGCATGTACATCTTCAACCCCTCCGTCCTGAAGCGCATCGAGCTTCGCCCCACGTCGATCGA
GAAGGAGACGTTCCCCGCCATGGTTGCCGACAACCAGCTGCACTCGTTCGATCTCGAGGGCTTC
TGGATGGACGTTGGCCAGCCCAAGGACTTCCTCAGCGGCACCTGCCTGTACCTGTCCTCCCTCA
CCAAGAAGGGCAGCAAGGAGCTGACCCCTCCCACCGAGCCCTACGTTCACGGCGGCAACGTCAT
GATTCACCCTTCGGCCAAGATTGGAAAGAACTGCAGAATAGGCCCCAATGTCACCATTGGCCCG
GATGTTGTCGTCGGTGACGGCGTCCGCCTGCAGCGATGCGTCCTCCTCAAGGGCTCCAAGGTCA
AGGACCACGCCTGGGTCAAGTCGACGATTGTTGGCTGGAACAGCACCGTCGGTCGCTGGGCCCG
TCTCGAGAATGTGACTGTTCTCGGTGACGACGTGACCATTGGCGACGAGATTTACGTCAACGGC
GGCAGCGTCCTGCCTCACAAGTCCATCAAGGCCAACGTTGACGTTCCCGCCATCATTATGTGA*T*
TTATCTCATGTTGTCACGCATCCTTGGCTCGCATGGGCGTTTTTGTTCCCCATGCGCTGCTTTC
CGAGATGATCTTTGTTTCTTCTTCAAACCCCATCTTTTCTTCTTTTAACTTGACATTTCTCTTT
TTTTTTTTTTTTTCCTTTTACAGAACCCCATTTACGCCTTACCGCAAACTCACCACTCCTCCGC
TATTCTCAAGAGATACCCTATATTGGTGGGGGAAACAGTCTTTGAGAGAAAAGAAAACCAAGCC
ACATTTTATATAATTACTACTAGTCTCGACATCTTTTTTCCCTTTCTTCTTCTTCCTCAAGAAA
AAAGATGTCGTGTACACTTATGTTGAGCCCCAAGTAAATCGTTTGGCGTCTCGGGGAACCGGTT
GGCAAAGCATTCTTGGAGGGACAGGGACGAGGGCTGAGGGTTGAGAAGAGCAATGACGGACGAG
*GCACTCAAGATTTCCATGTATGAAAAGATGATAGCGTAGCGAATGAAGTGTATTTACGCTTG*CG
CCGACTGTGTTGTCTGGTGACGCGATTGCTGAGGTCGAGCTTGTCCAGTACGAGCACTGCTTGA
AGATGAACAAATCGAGGTGGTTCCCCCATAGGCTGACCTTATACAGAATTTCGCTATGCATCAG
AAGTAAGTCGTTATCACATTTGATGAGATAGCATCTCCGCTCACTTGTCATTTCAGTTAGAATA
TTCATT

[0131] As shown in Table 1, MAGI 10-8g has a reduction in broth viscosity compared to the parent MAGI. At the end of the batch growth phase, when all the glucose has been consumed, both strains had achieved a similar biomass concentration. To get there, the MAGI control strain saw agitation increased to the maximum of 750 rpm and then saw DO drop down to as low as 35%. The strain MAGI 10-8g did not require as much energy to achieve the same biomass concentration. Agitation rate was increased slightly to 513 rpm when the % DO dropped to 40. Protein production was not adversely affected in MAGI 10-8g compared to MAGI (not shown).

Table 1. Broth viscosity of MAGI compared to MAGI 10-8 g

Strain	Deletion	DO (%)	Agitation (rpm)	Biomass (g/kg)	CER (mmol/L/hr)
MAGI	none	35	750	39	125
MAGI 10-8g	*mpg1*	40	513	40	128

[0132] Inverse PCR was used to identify the insertion site of the T-DNA containing the *pyr2 his1* genes in the *T. reesei* genome. Briefly, high molecular-weight genomic DNA from strain MAGI 10-8g was digested to completion with the restriction enzyme *Spe*I. After heat inactivation of the enzymes, the reaction was diluted five-fold in ligation buffer and T4 DNA ligase was added. Following an overnight ligation reaction, the ligase was heat inactivated and the reaction was precipitated with ammonium acetate and ethanol. The washed DNA pellet was dissolved in TE and used as template for PCR with primers RPG253 and RPG255 (referring to Table 2). The resulting PCR product was cleaned then sequenced with nested primers RPG239 and RPG207 to determine the nucleotide sequence flanking the site of the T-DNA insertion. BLASTn analysis of this sequence against the JGI *Trichoderma reesei* v 2.0 genome sequence revealed that the T-DNA had deleted the region 369089 to 370324 of Scaffold 13.

[0133] The site of insertion was confirmed by PCR using primers homologous to the genomic DNA flanking the insertion site and primers homologous to the T-DNA. In particular, primers RPG256 and RPG268 were used to confirm the sequence at the 3′ end of the T-DNA and primers RPG268 and RPG269 amplified the full T-DNA insertion at the identified site.

[0134] The site of the T-DNA insertion in mutant MAGI 10-8g was at Scaffold 13 from 369089 to 370324 in the *T. reesei* JGI genomic database v2. The gene found at this site is the *mpg1* gene (PDI 122551) which is found in other fungi including *Aspergillus clavatus, Aspergillus fumigatus, and Neosartorya fischeri*. As described by Kruszewska *et al.* (1998) *Cur. Genet.* 33:445-50 and Zakrzewska *et al.* (2003) *Applied and Environmental Microbiology* 69:4383-4389 *mpg1* from *Trichoderma reesei* encodes for a GTP:alpha-D-mannose-1-phoshate guanyltransferase which can play a major regulatory role in early stages of protein glycosylation. Southern analysis showed that this strain contained only one copy of the *pyr2* gene in addition to the native copy indicating that one disruption event had taken place (not shown).

[0135] Since the insertion at this site was shown to be the only genetic change made in the MAGI 10-8g strain, it follows that disruption of the *mpg1* gene was responsible for the observed morphological changes.

Table 2. Primers used in Example 1.

Primer	Sequence	SEQ ID NO
RPG253	5′- TTCCTGACAACGAGGACATCTCAAGCTGT-3′	17
RPG255	5′- CAAACATAGCAGCGTCCATTGCACGA-3′	18
RPG239	5′- GGGGACAAGTTTGTACAAAAAAGCAGGCTTGATGG-TTGACTATTGGGTTTCTGTGC-3′	19
RPG207	5′- GTCGCCCGTCTCCGTTGT-3′	20
RPG256	5′- GCTTTCGAGCTCACACGACATCCTTCA-3′	21
RPG268	5′- TCCCCGAGACGCCAAACGA-3′	22
RPG269	5′- GGCCGAGGACCCTTCCATCA-3′	23

Example 2. Deletion of the *mpg1* gene from *T. reesei* mutant 77B7

A. Morph strain TrGA 77B7

[0136] The Morph strain, described above, was previously transformed with a native *Trichoderma* glucoamylase gene (TrGA) under control of the CBH1 promoter, using *amds* as a marker. A transformant containing two tandem copies of glucoamylase (TrGA 29-9) was subsequently isolated, and random chemical mutagenesis was used to produce a mutant (77B7). A spontaneous *pyr2* mutant derivative was subsequently isolated by 5-fluoro-orotic acid (FOA) selection.

B. Generation of a *mpg1* disruption cassette

[0137] The Trichoderma *reesei mpg1* ((jgi|Trire2|122551) was deleted from mutant Morph 77B7.

[0138] The *mpg1* disruption cassette plasmid pRATT249 (Figure 2) was prepared using standard molecular biology procedures. This plasmid included a DNA sequence having a 2.5 Kb region homologous to the DNA sequence spanning part of the 5′ untranslated region and contiguous upstream sequences (Left Flank). Also included within the plasmid was a DNA sequence having a 3.3 Kb region homologous to the DNA sequence spanning part of the fourth exon of the *mpg1*gene and contiguous downstream sequences (Right Flank). These sequences were designed to target the *mpg1* gene and replace the regions of the genome between the Left and Right Flanks with the intervening cassette sequences. These intervening sequences included a *pyr2* selection marker from *Trichoderma atroviride* intended to minimize homology to the endogenous *T. reesei pyr2* in the genome of the strain to be transformed. Immediately upstream of the *pyr2* selection marker was a directly repeated duplication of the 3′end of the marker, which facilitated the subsequent loss of the marker and isolation of useful *pyr2* mutant derivatives of the transformants/disruptants. This full *mpg1* disruption cassette was amplified by

PCR using primers RPG388 and RPG391. Multiple PCR reactions were pooled and cleaned using standard molecular biology procedures for use in the subsequent steps.

C. Generation of strain Morph 77B7Δ*mpg1*

[0139] Strain Morph TrGA 77B7 Δ *pyr2* was transformed with the *mpg1* disruption cassette using PEG-mediated transformation, and plated on Vogel's minimal medium containing sorbitol to select for candidates based on uridine prototrophy acquired by the *pyr2* marker. Individual transformants were isolated and propagated by transfer to Vogel's minimal medium. PCR analysis was used to identify transformants in which the *mpg1* disruption cassette integrated at the *mpg1* locus by homologous recombination. Homologous integration of the Δ*mpg1* disruption cassette at the *mpg1* locus was verified by amplifying DNA fragments of the expected sizes using two primer pairs. Primer pair RPG394 and RPG253 amplified a DNA fragment starting outside the 5′ end of the disruption cassette region and ending within the 3′ region. Primer pair RPG395 and RPG273 amplified a DNA fragment starting within the 5′ region of the disruption cassette and ending outside the 3′ end of the disruption cassette region. The generated strain with confirmed homologous integration of the *mpg 1* disruption cassette was named Morph 77B7 Δ *mpg1*. Primer sequences are listed in Table 4

[0140] Strains Morph 77B7 and Morph 77B7 Δ *mpg1* were grown under identical conditions in submerged (liquid) culture, and their growth phenotypes were compared. Briefly, spores of each strain were added separately to 500-mL of medium in a 3-L flask with both side and bottom baffles. The medium contained 5 g/L $(NH_4)_2SO_4$, 4.5 g/L KH_2PO_4, 1 g/L $MgSO_4 \cdot 7 \cdot H_2O$, and 14.4 g/L citric acid, adjusted to pH 5.5 with 5% NaOH. After autoclaving for 30 minutes, sterile 60% glucose was added to a final concentration of 27.5 g/L, along with 2.5 mL/L of a trace element solution containing 175 g/L citric acid, 200 g/L $FeSO_4 \cdot 7 \cdot H2O$, 16 g/L $ZnSO_4 \cdot 7 \cdot H_2O$, 3.2 g/L $CuSO_4 \cdot 5 \cdot H_2O$, 1.4 g/L $MnSO_4 \cdot H_2O$, and 0.8 g/L H_3BO_3. The culture was grown for 48 hrs at 34°C in a shaking incubator.

[0141] After 48 hrs, the contents of each flask were added separately to 14-L fermentors containing 9.5 L of medium containing 4.7 g/L KH_2PO_4, 1.0 g/L $MgSO_4 \cdot 7 \cdot H_2O$, 4.3 g/L $(NH_4)_2SO_4$ and 2.5 mL/L of the same trace element solution. These components were heat sterilized together at 121°C for 30 minutes. A solution of 60% glucose and 0.48% $CaCl_2 \cdot 2 \cdot H_2O$ was separately autoclaved, cooled, and added to the fermentor to a final concentration of 75 g/L glucose and 0.6 g/L $CaCl_2 \cdot 2 \cdot H_2O$. The medium was adjusted to pH 3.5 with 28% NH_3 and the temperature was maintained at 34°C for the entire growth period.

[0142] A dissolved oxygen (DO) probe was calibrated to 100% when there was no added pressure in the headspace (*i.e.*, 0 bar gauge, 1 bar absolute). The pressure in the headspace was

then set to 0.7 bar (gauge), after which the oxygen probe read 170% before the seed culture was added. The fermentor contained two, four-blade turbines that provided mixing via a variable speed motor that was initially set at 500 rpm.

[0143] As the cultures grew, DO levels dropped, at least partly as a consequence of the increased viscosity of the broth due to the proliferation of filamentous fungus hyphae. When DO fell below 40%, the agitation rate was increased to maintain the dissolved oxygen at 40%. Upon reaching 750 rpm agitation, DO would be allowed to drop below 40%. If the DO did not fall below 40%, then it was unnecessary to increase the agitation rate during the fermentation run, and the initial agitation rate was higher than necessary. When the glucose was completely consumed, the amount of biomass produced in each fermentor was measured, and found to be substantially the same for all both strains.

[0144] The DO level in each fermentor at a given level of agitation, and the amount of agitation required to maintain a given DO level are indirect measures of the viscosity of the different broths, due to the different strain growth phenotypes. Although it would be ideal to vary only one variable (*i.e.*, DO or agitation) and measure the other, it is desirable to prevent the DO from falling below 40% to ensure the production of sufficient biomass in each fermentor, thereby permitting a more meaningful comparison between the growth of the different strains.

[0145] Generally, where it is necessary to increase the agitation rate to maintain a target DO level, the amount of agitation can be estimated by the amount of power supplied to the motor driving the fermentor turbine, which provides a metric that correlates with the viscosity of the broth. In particular, the extra power required to agitate the suspended culture is proportional to the agitation rate raised to the 3rd power.

[0146] For strains where the %DO does not fall below 40%, the metric is based on the minimal dissolved oxygen levels that were maintained at the preselected agitation rate),

[0147] As shown in Table 3, deletion of the *mpg1*gene from strain Morph 77B7 resulted in a strain (Morph 77B7 Δ *mpg1*) having a reduction in broth viscosity. At the end of the batch growth phase, when all the glucose has been consumed, both strains had achieved a similar biomass concentration. To get there, the control strain saw agitation increased to 616 rpm when the DO drop down to as low as 40%. The *mpg1*-deleted strain did not require as much energy to achieve the same biomass concentration. Agitation rate was never increased above 500 rpm and DO dropped only as low as 102%.

Table 3. Broth viscosity in Morph 77B7 with and without the *mpg1* gene

Strain	Deletion	DO (%)	Agitation (rpm)	Biomass (g/kg)	CER (mmol/L/hr)
Morph77B7	None	40	616	40	141
Morph 77B7Δ*mpg1*	*mpg1*	102	500	42	118

Table 4. Primers used in Example 2

Primer	Sequence	SEQ ID NO
RPG388	5′- CCCCTCCGGATGAGGTGGCTTGTGGCT-3′	24
RPG391	5′- GGCGGCTAGCAGACGCACTCGTAGAGCAAGGT-3′	25
RPG394	5′- AGGTCCGATCAACGACTCTGGCAAC-3′	26
RPG253	5′- TTCCTGACAACGAGGACATCTCAAGCTGT-3′	27
RPG395	5′- GGGTTGTCGTTAGCTAACCAGAGCGTAA-3′	28
RPG273	5′- GGTCAGTAACATAGCAGGACTATAGTAGTGGCTCAC-3′	29

Example 3. Additive viscosity reduction in mutants having disrupted *mpg1* and *seb1* genes

[0148] A. Morph 77B7 Δ *mpg1*, described above, was previously transformed with a native *Trichoderma* glucoamylase gene (TrGA) under control of the CBH1 promoter, using *amdS* as a marker. A transformant containing two tandem copies of glucoamylase (TrGA 29-9) was subsequently isolated, and random chemical mutagenesis was used to produce mutant (77B7) having altered morphology associated with a low viscosity phenotype. The *mpg1* gene was deleted as described above. The *pyr2* gene was subsequently spontaneously deleted by selecting for resistance to 5-fluoroorotic acid creating strain Morph 77B7 Δ *mpg1*, Δ*pyr2*.

B. Generation of a *seb1* disruption cassette

[0149] The *seb1* disruption cassette plasmid pRATT240 (Figure 3) was prepared using standard molecular biology procedures. These intervening sequences included a *pyr2* selection marker from *Trichoderma atroviride* intended to minimize homology to the endogenous *T. reesei pyr2* in the genome of the strain to be transformed. Immediately upstream of the *pyr2* selection marker was a duplication of the 3′end of the marker, which direct repeat facilitated the subsequent loss of the marker and isolation of useful *pyr2* mutant derivatives of the transformants/disruptants. This full *seb1* disruption cassette was amplified by PCR using primers RPG257 and RPG264 (referring to Table 6). Multiple PCR reactions were pooled and cleaned using standard molecular biology procedures for use in the subsequent steps.

C. Generation of strain Morph 77B7 Δ *mpg1*Δ*seb1* and Morph 77B7 Δ *seb1*

[0150] Morph 77B7 Δ*pyr2* and Morph 77B7 Δ*mpg1* Δ*pyr2* were transformed with the *seb1* disruption cassette using PEG-mediated transformation, and plated on Vogel's minimal medium containing sorbitol to select for candidates based on uridine prototrophy acquired by the *pyr2* marker. Individual transformants were isolated and propagated by transfer to Vogel's minimal medium. PCR analysis was used to identify transformants in which the *seb1* disruption cassette integrated at the *seb1* locus by homologous recombination. Homologous integration of the Δ*seb1* disruption cassette at the *seb1* locus was verified by amplifying DNA fragments of the expected sizes using two primer pairs. Primer pair RPG297 and RPG253 amplified a DNA fragment starting outside the 5′ end of the disruption cassette region and ending within 3′ region. Primer pair RPG296 and RPG273 amplified a DNA fragment starting within the 5′ region of the disruption cassette and ending outside the 3′ end of the disruption cassette region. Consistent with disruption, a third primer pair, RPG133 and RPG220, amplified a 1.6 kb DNA fragment spanning the insertion site using template DNA from the untransformed parental strain but failed to amplify this fragment using template DNA from the *seb1* disruption strain. The generated strains with confirmed homologous integration of the *seb1* disruption cassette was named Morph 77B7 Δ*seb1* and Morph 77B7 Δ *mpg1* Δ *seb1*.

D. Growth of Morph 77B7 Δ *mpg1* Δ*seb1* in submerged culture

[0151] Strains Morph 77B7 Δ *mpg1* and Morph 77B7 Δ *mpg1* Δ*seb1* were grown under identical conditions in submerged (liquid) culture as described in Example 2, and their growth phenotypes were compared. As shown in Table 5, disruption of the *seb1* gene in the Morph 77B7 Δ *mpg1* strain resulted in a strain having a further reduction in viscosity (based on the minimal maintained dissolved oxygen levels at the preselected agitation rate), indicating that disruption of the *seb1* gene and disruption of the *mpg1* gene have an additive effect with respect to morphology and viscosity reduction. Protein production of Morph 77B7 Δ*mpg1* Δ*seb1* was at least 85% or higher of that of Morph TrGA 77B7 and Morph TrGA 77B7Δ*seb1*.

Table 5. Broth viscosity of Morph 77B7 Δ *seb1*, Morph 77B7 Δ*mpg1*, and Morph Δ *mpg1* Δ*seb1*

Strain	Deletion	DO (%)	Agitation (rpm)	Biomass (g/kg)	CER (mmol/L/hr)
Morph 77B7Δ*seb1*	*seb1*	101	500	41	127
Morph 77B7 Δ*mpg1*	*mpg1*	102	500	42	118
Morph TrGA 77B7Δ*mpg1*Δ*seb1*	*mpg1*, *seb1*	110	500	47	112

Table 6. Primers used in Example 3.

Primer	Sequence	SEQ ID NO
RPG257	5′-AGATACTAGTGCGAGGCATCCGTGATGGATCTC-3′	30
RPG264	5′-GGGTCCCGGGCTCGGGAGCGTAACTCTTGTCC-3′	31
RPG297	5′-CGCCGTCAGTTGACGACAGTGCT-3′	32
RPG253	5′-TTCCTGACAACGAGGACATCTCAAGCTGT-3′	33
RPG296	5′-CACCGGTGAAGCCTTCCGTGAGT-3′	34
RPG273	5′-GGTCAGTAACATAGCAGGACTATAGTAGTGGCTCAC-3′	35
RPG133	5′-GGAGCCAACAGAGACGGTCAGGTT-3′	36
RPG220	5′-GCCCAGCGTCGAGTGAGACAAGT-3′	37

Example 4. Additive viscosity reduction in mutants having disrupted *mpg1* and *sfb3* genes

A. Morph strain TrGA #32

[0152] The Morph strain, described above, was previously transformed with a native *Trichoderma* glucoamylase gene (TrGA) under control of the CBH1 promoter, using *amds* as a marker. A transformant containing two tandom copies of glucoamylase (TrGA 29-9) was subsequently isolated, and random chemical mutagenesis was used to produce a cell wall mutant (70H2) having altered morphology associated with a reduced viscosity phenotype. This reduced viscosity phenotype was later determined to be the result of a truncated *sfb3* gene (data not shown). A 70H2 strain transformed with additional copies of TrGA (*i.e.*, TrGA #32) has further been useful for over-expressing TrGA.

B. Generation of a *mpg1* disruption cassette

[0153] The *mpg1* gene was disrupted as described in Example 2 to make strain TrGA #32 Δ*mpg1*.

C. Growth of TrGA#32 Δ*mpg1* in submerged culture

[0154] Strains TrGA#32 and TrGA#32 Δ*mpg1* were grown under identical conditions in submerged (liquid) culture as described in Example 2, and their growth phenotypes were compared. As shown in Table 7, deletion of the *mpg1* gene from the TrGA#32 strain resulted in a strain having a further reduction in viscosity (based on the rpm required to maintain a preselected level of dissolved oxygen), indicating that disruption of the *mpg1* gene and disruption of the *sfb3* gene have an additive effect with respect to morphology and viscosity reduction. Protein production was not affected by the *mpg1* deletion (not shown).

Table 7. Growth characteristics of TrGA #32 and TrGA #32 Δ*mpg1* in liquid medium.

Strain	Deletion(s)	DO (%)	Agitation (rpm)	Biomass (g/kg)	CER (mmol/L/hr)
TrGA #32	*sfb3*	40	618	38	147
TrGA #32 *Δmpg1*	*sfb3/mpg1*	40	589	41	153

Example 5. Additive viscosity reduction in mutants having disrupted at least one of *gas1, crz1* and *tps2* genes in conjunction with disrupted *mpg1, seb1*, and/or *sbf3*

A. Viscosity reduction in disrupted *gas1*

[0155] The Gel/Gas/Phr family of fungal β(1,3)-glucanosyltransferases plays an important role in cell wall biogenesis by processing the main component β(1,3)-glucan (Popolo *et* al., 2008). *gas1* (PID 22914) encodes a beta-1,3-glucanosyltransferase that is a GPI (and/or glucan)-anchored protein capable of breaking and joining beta-1,3-glucans. There are multiple paralogs in many fungal genomes including *T. reesei,* which has five. Separate studies have shown that mutation of the *gas1* gene (or the *gel1* gene as it is known in *Aspergillus fumigatus*) affects fungal cell wall structure, and can lead to morphological changes as well as hypersensitivity to Calcofluor White, Congo Red and sodium dodecyl sulfate (Schirawski, J. *et al.* 2005, Mouyna, I. *et al.* 2005).

[0156] A *Trichoderma reesei* Morph strain was deleted for four major cellulase genes, including *cbhI, cbhII, egII,* and *egIV,* which makes it particular suitable for expressing other proteins in the absence of or in reduced cellulase background. *See,* WO 05/001036. The Morph strain had been previously transformed with a native *Trichoderma* glucoamylase gene (TrGA) under control of the CBH1 promoter, using *amdS* as a marker. A transformant containing two tandem copies of glucoamylase (TrGA 29-9) was subsequently isolated, and random chemical mutagenesis was used to produce a mutant (77B7). A spontaneous *pyr2* mutant derivative was subsequently isolated by *5-fluoro*-orotic acid (FOA) selection. The *Trichoderma reesei gas1* (PID 22914) was deleted from mutant Morph 77B7.

[0157] Strain Morph TrGA 77B7 Δ*pyr2* was transformed with a *gas1* disruption cassette using PEG-mediated transformation, and plated on Vogel's minimal medium containing sorbitol to select for candidates based on uridine prototrophy acquired by the *pyr2* marker. As shown in Table 8, Morph 77B7 Δ*gas1* has a reduction in broth viscosity compared to the parent Morph 77B7. At the end of the batch growth phase, when all the glucose has been consumed, both strains had achieved a similar biomass concentration. To arrive at the end of the batch growth phase, the Morph 77B7 control strain saw agitation increased to 616 rpm and then saw DO

content level drop down to as low as 40%. The strain Morph 77B7 Δ*gas1* did not require as much energy (*i.e.*, rpm increase in agitation) to achieve the same biomass concentration. Agitation rate never increased above 500 rpm and the % DO never dropped below 115. Protein production was not adversely affected in Morph 77B7 Δ*gas1* compared to Morph 77B7 (data not shown). Details of the *gas1* disruption can be found in U.S. Provisional Application No. 61,480,602, filed April 29, 2011.

Table 8. Broth viscosity of Morph 77B7 compared to Morph 77b7 Δ*gas1*

Strain	Deletion	DO (%)	Agitation (rpm)	Biomass (g/kg)	CER (mmol/L/hr)
Morph 77b7	none	40	616	38	141
Morph 77b7Δ*gas1*	*gas1*	115	500	39	147

B. **Viscosity reduction in disrupted *crz1***

[0158] In fungi, calcineurin mediated Ca^{2+} signaling has been shown to be required for growth, development, and virulence in many organisms. It is necessary for adaption to diverse environmental conditions including high cation levels and alkaline pH. The gene *crz*1 encodes a calcineurin-regulated transcription factor. The Crz1p transcription factor is dephosphorylated when the phosphatase calcineurin is activated by Ca^{2+}/calmodulin. It then enters the nucleus and induces expression of a number of genes, many of which encode proteins with cell wall-related functions (Yoshimoto *et al.*, 2002; Lagorce *et al.*, 2003; Garcia *et al.*, 2004; Karababa *et al.*, 2006; Pardini *et al.*, 2006, Munro, C. *et al.* 2009). Deletion of *crz*1 or a homolog can result in alterations in hyphal morphology (Kothe, G. and Free, S. 1998, Prokisch, H. *et al.* 1997).

[0159] A *Trichoderma reesei* Morph strain was prepared as described above. The *Trichoderma reesei crz1* (PID 36391) was deleted from mutant Morph 77B7. Strain Morph TrGA 77B7 Δ*pyr2* was transformed with the *crz1* disruption cassette using PEG-mediated transformation, and plated on Vogel's minimal medium containing sorbitol to select for candidates based on uridine prototrophy acquired by the *pyr2* marker. As shown in Table 9, Morph 77B7 Δ*crz1* has a reduction in broth viscosity compared to the parent Morph 77B7. At the end of the batch growth phase, when all the glucose has been consumed, both strains had achieved a similar biomass concentration. To arrive at the end of the batch growth phase, the Morph 77B7 control strain saw agitation increased to 616 rpm and then saw DO content level drop down to as low as 40%. The strain Morph 77B7 Δ*crz1* did not require as much energy to achieve the same biomass concentration. Agitation rate never increased above 500 rpm and the % DO never

dropped below 100. Details of the *crz1* disruption can be found in U.S. Provisional Application No. 61,480,610, filed April 29, 2011.

Table 9. Broth viscosity of Morph 77B7 compared to Morph 77b7 Δ*crz1*

Strain	Deletion	DO (%)	Agitation (rpm)	Biomass (g/kg)	CER (mmol/L/hr)
Morph 77b7	none	40	616	38	141
Morph 77b7Δ*crz1*	*crz1*	100	500	39	120

C. **Viscosity reduction in disrupted *tps1***

[0160] The gene *tps2* encodes a trehalose-phosphate phosphatase involved in the synthesis of the disaccharide trehalose. Trehalose is a stress induced sugar that buffers the refolding of denatured proteins in the cytoplasm and ER (Singer, M *et al.* 1998, Simola, M *et al.* 2000). This disaccharide is produced in large quantities by diverse organisms in response to a variety of stresses. In yeast, trehalose stabilizes proteins at high temperatures and assists in refolding heat damaged proteins (Simola, M *et al.* 2000).

[0161] A *Trichoderma reesei* Morph strain was prepared as described above. The *Trichoderma reesei tps2* (PID 48707) was deleted from mutant Morph 77B7. Strain Morph TrGA 77B7 Δ*pyr2* was transformed with the *tps2* disruption cassette using PEG-mediated transformation, and plated on Vogel's minimal medium containing sorbitol to select for candidates based on uridine prototrophy acquired by the *pyr2* marker. As shown in Table 10, Morph 77B7 Δ*tps2* has a reduction in broth viscosity compared to the parent Morph 77B7. At the end of the batch growth phase, when all the glucose had been consumed, both strains had achieved a similar biomass concentration. To arrive at the end of the batch growth phase, the Morph 77B7 control strain saw agitation increased to 616 rpm and then saw DO content level drop down to as low as 40%. The strain Morph 77B7 Δ*tps2* did not require as much energy to achieve the same biomass concentration. Agitation rate never increased above 500 rpm and the % DO never dropped below 110. Details of the *tps1* disruption can be found in U.S. Provisional Application No. 61,480,629, filed April 29, 2011.

Table 10. Broth viscosity of Morph 77B7 compared to Morph 77b7 Δ*tps2*

Strain	Deletion	DO (%)	Agitation (rpm)	Biomass (g/kg)	CER (mmol/L/hr)
Morph 77b7	none	40	616	38	141
Morph 77b7Δ*tps2*	*tps2*	110	500	41	94

[0162] Although the foregoing compositions and methods have been described in some detail by way of illustration and examples for purposes of clarity of understanding, it will be apparent to those skilled in the art that certain changes and modifications can be made. Therefore, the description should not be construed as limiting the scope of the invention, which is delineated by the appended claims.

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[0164] The following references, and additional reference are cited herein:

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5

CLAIMS

What is claimed is:

1. A variant strain of *Trichoderma sp.* fungus derived from a parental strain, the variant strain comprising a genetic alteration that causes cells of the variant strain to produce a reduced amount of functional GTP:alpha-D-mannose-1-phoshate guanyltransferase (Mpg1) protein compared to cells of the parental strain, wherein the cells of the variant strain produce during aerobic fermentation in submerged culture a cell broth that (i) requires a reduced amount of agitation to maintain a preselected dissolved oxygen content compared to the cells of the parental strain, and/or (ii) maintains an increased dissolved oxygen content at a preselected amount of agitation, compared to the cells of the parental strain.

2. The variant strain of claim 1, wherein the genetic alteration comprises a disruption of the GTP:alpha-D-mannose-1-phoshate guanyltransferase (*mpg1*) gene present in the parental strain.

3. The variant strain of claim 2, wherein disruption of the *mpg1* gene is the result of deletion of all or part of the *mpg1* gene.

4. The variant strain of claim 2, wherein disruption of the *mpg1* gene is the result of deletion of a portion of genomic DNA comprising the *mpg1* gene.

5. The variant strain of claim 2, wherein disruption of the *mpg1* gene is the result of mutagenesis of the *mpg1* gene.

6. The variant strain of any one of claims 2-5, wherein disruption of the *mpg1* gene is performed using site-specific recombination.

7. The variant strain of any one of claims 2-6, wherein disruption of the *mpg1* gene is performed in combination with introducing a selectable marker at the genetic locus of the *mpg1* gene.

8. The variant strain of any one of claims 1-7, wherein the variant strain does not produce functional Mpg1 protein.

9. The variant strain of any one of claims 1-7, wherein the variant strain does not produce Mpg1 protein.

10. The variant strain of any one of claims 1-9, wherein the variant strain further comprises a gene encoding a protein of interest.

11. The variant strain of any one of claims 1-10, wherein the variant strain produces substantially the same amount of, or more, protein per unit amount of biomass as the parental strain.

12. The variant strain of any one of claims 1-11, wherein the filamentous fungus is *Trichoderma reesei.*

13. A method for producing a variant strain of a *Trichoderma sp.* fungus cell comprising: introducing a genetic alteration into a parental strain of a filamentous fungal cell, which genetic alteration reduces the production of functional GTP:alpha-D-mannose-1-phoshate guanyltransferase (Mpg1) protein compared to the cells of the parental strain, thereby producing a variant filamentous fungal cell that produces during aerobic fermentation in submerged culture a cell broth that (i) requires a reduced amount of agitation to maintain a preselected dissolved oxygen content, compared to the cells of the parental strain, and/or (ii) maintains an increased dissolved oxygen content at a preselected amount of agitation, compared to the cells of the parental strain.

14. The method of claim 13, wherein the genetic alteration comprises disrupting the GTP:alpha-D-mannose-1-phoshate guanyltransferase (*mpg1)* gene in the parental filamentous fungal cell using genetic manipulation.

15. The method of claim 13 or 14, wherein the genetic alteration comprises deleting the GTP:alpha-D-mannose-1-phoshate guanyltransferase (*mpg1)* gene in the parental filamentous fungal cell using genetic manipulation.

16. The method of any one of claims 13-15, wherein the genetic alteration is performed using site-specific genetic recombination.

17. The method of any one of claims 13-16, wherein disruption of the *mpg1* gene is performed in combination with introducing a selectable marker at the genetic locus of the *mpg1* gene.

18. The method of any one of claims 13-17, wherein the variant strain produces substantially the same amount of, or more, protein per unit amount of biomass as the parental strain.

19. The method of any one of claims 13-18, wherein the *Trichoderma sp.* fungus cell is *Trichoderma reesei*.

20. The method of any one of claims 13-19, wherein the parental strain further comprises a gene encoding a protein of interest.

21. The method of claim 20, wherein the gene encoding the protein of interest is present in the parental strain prior to introducing the genetic alteration that reduces the production of functional Mpg1 protein.

22. The variant strain of the *Trichoderma sp.* fungus cell produced by the method of any one of claims 13-21, wherein the cells of the variant strain produce during aerobic fermentation in submerged culture a cell broth that (i) requires a reduced amount of agitation to maintain a preselected dissolved oxygen content compared to the cells of the parental strain, and/or (ii) maintains an increased dissolved oxygen content at a preselected amount of agitation, compared to the cells of the parental strain.

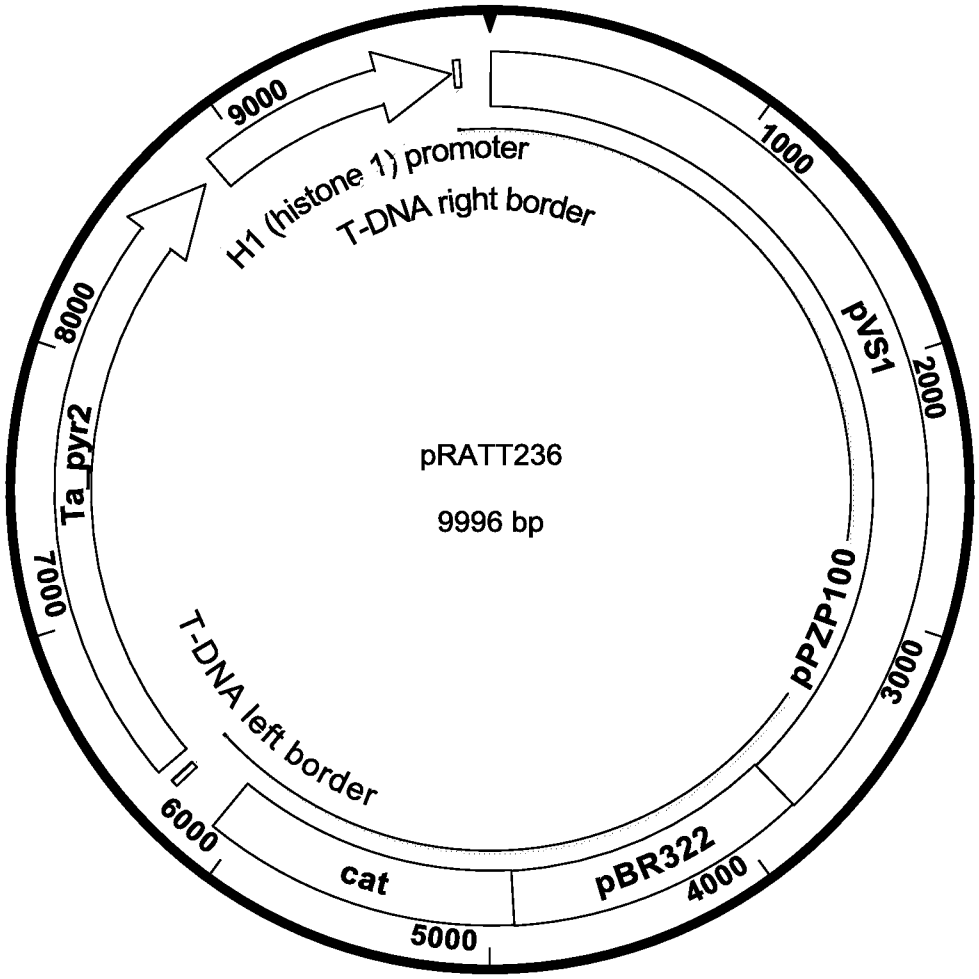
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Figure 1

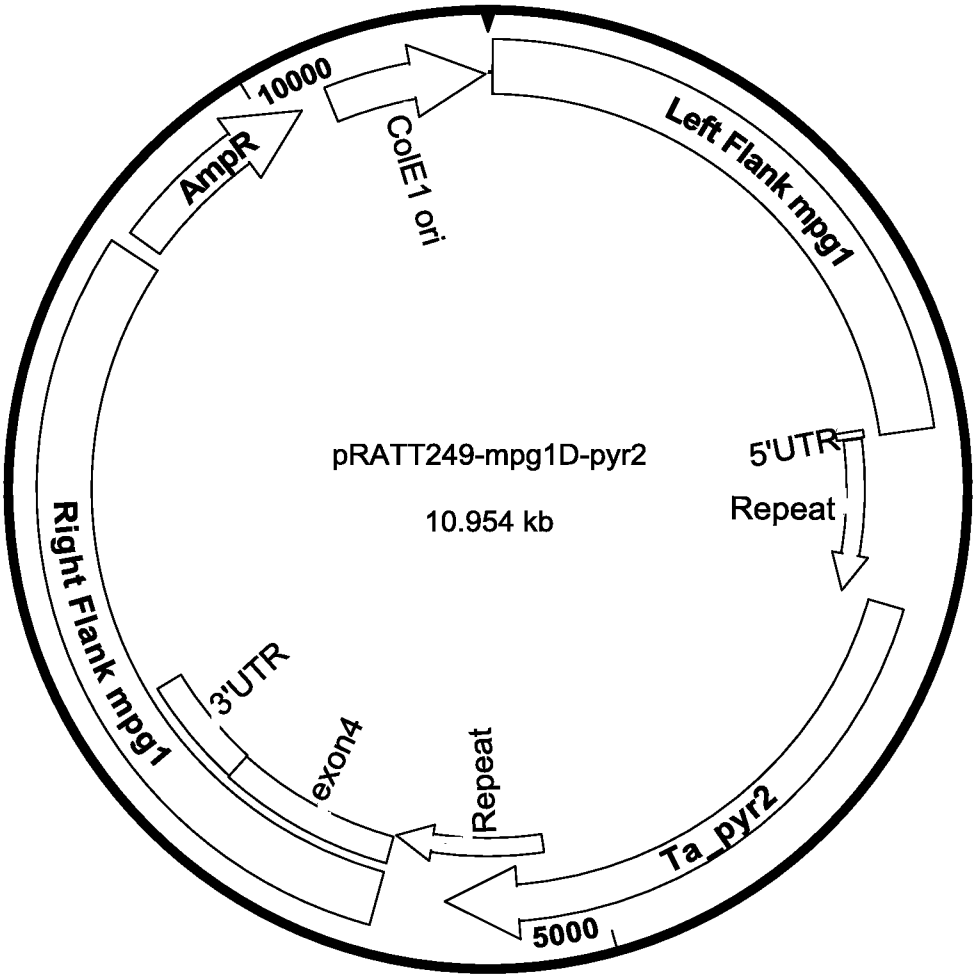
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Figure 2

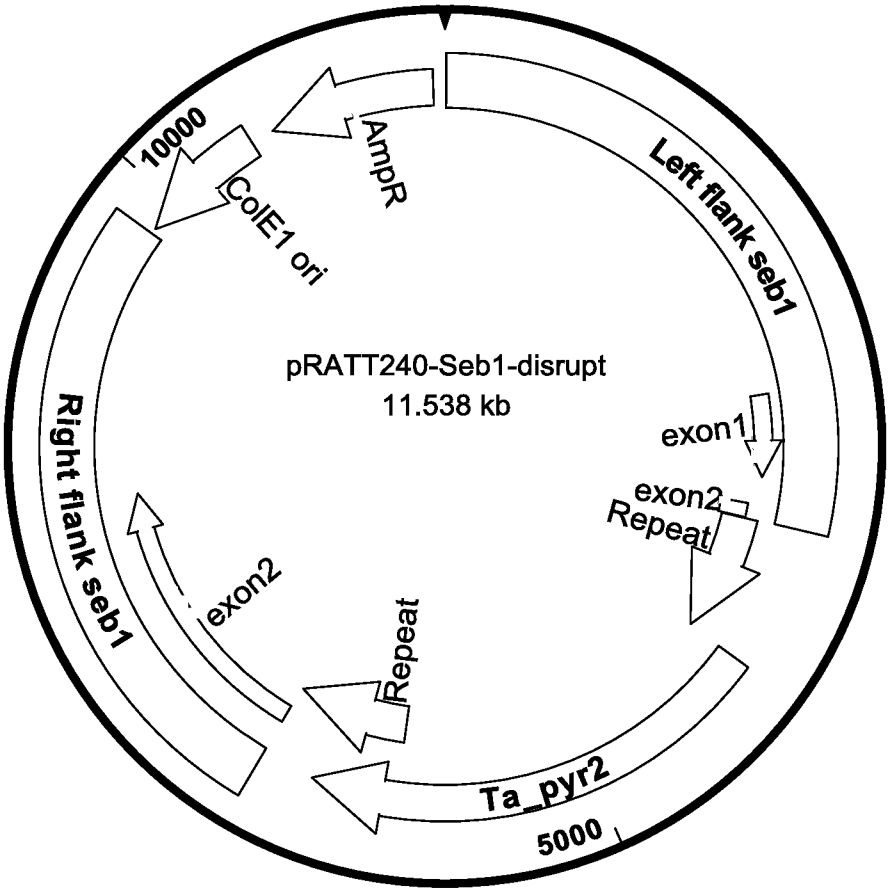
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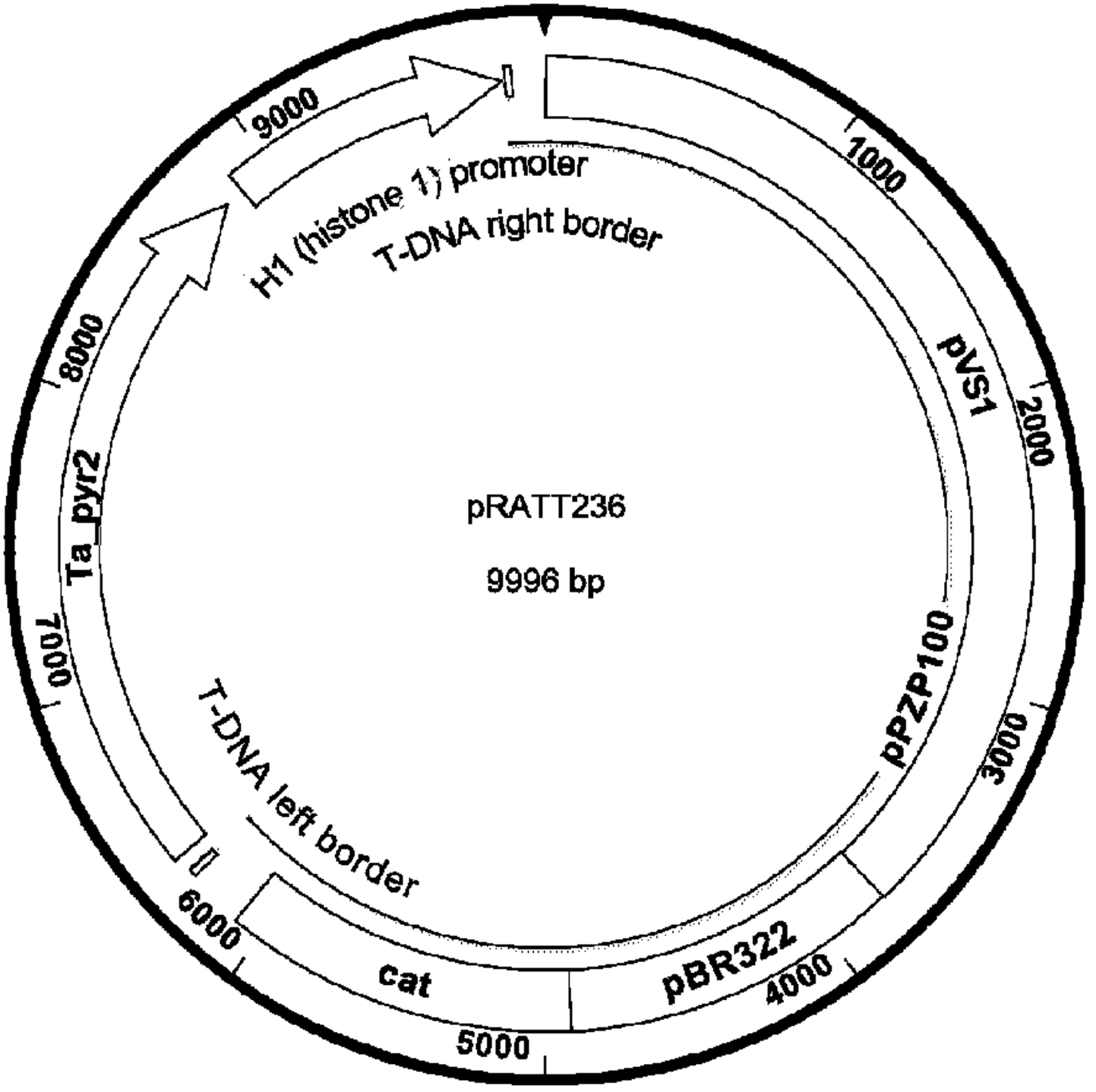
Figure 3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