Zakrzewska et al (Curr Genet 2003 43: 11-16) further reported that *Trichoderma reesei pmt1* gene did not functionally complement pmt4Δ *S. cerevisiae* mutant.

In fact, deletions of the PMT genes in yeasts or filamentous fungi appears to either result in no phenotype at all or lethality or severely impaired vital functions of the cells, which would not be suitable for recombinant production of heterologous proteins, especially mammalian glycoproteins. For this reason, alternative methods such as the use of pmt inhibitors have been proposed as an alternative to pmt knock out strains (WO2009/143041).

Thus, a need remains for improved filamentous fungal cells, such as *Trichoderma* fungus cells, that can stably produce heterologous proteins with no or reduced O-mannosylation, such as immunoglobulins, preferably at high levels of expression.

SUMMARY

The present disclosure relates to improved methods for producing proteins with no or reduced O-mannosylation in filamentous fungal expression systems, and more specifically, glycoproteins, such as antibodies or related immunoglobulins or fusion proteins which may be O-mannosylated when produced in filamentous fungal expression systems.

The present disclosure is based in part on the surprising discovery that filamentous fungal cells, such as *Trichoderma* cells, can be genetically modified to reduce or suppress O-mannosylation activity, without adversely affecting viability and yield of produced glycoproteins.

Accordingly, in a first aspect, the disclosure relates to a PMT-deficient filamentous fungal cell comprising:

a) a first mutation that reduces or eliminates an endogenous protease activity compared to a parental filamentous fungal cell which does not have said first mutation, said endogenous proteases being selected among aspartic proteases, trypsin-like serine proteases, subtilisin proteases, glutamic proteases and sedolisin proteases, and,

b) a second mutation in a protein O-mannosyltransferase (*PMT*) gene that reduces endogenous O-mannosyltransferase activity compared to a parental filamentous fungal cell which does not have said second mutation,

wherein said filamentous fungal cell is selected from the group consisting of *Trichoderma*, *Neurospora*, *Myceliophthora* and *Chrysosporium* cell.

preceding embodiments, less than 1.0%, 0.5%, 0.1%, 0.01 %, 0.001 %, or 0 % of the antibody composition comprises glycation structures.

In another embodiment that may be combined with one or more of the preceding embodiments, the glycoprotein composition, such as an antibody is devoid of one, two, three, four, five, or six of the structures selected from the group of Neu5Gc, terminal Galα3Galβ4GlcNAc, terminal Galβ4GlcNAc, terminal Galβ3GlcNAc, core linked fucose and glycation structures.

The disclosure also relates to a method of reducing O-mannosylation level of a recombinant glycoprotein composition produced in a filamentous fungal cell, for example, *Trichoderma* cell, typically, *Trichoderma reesei*, said method consisting of using a filamentous fungal cell having a mutation in a *PMT* gene wherein said *PMT* gene is either:

i. *PMT1* gene comprising the polynucleotide of SEQ ID NO:1,
ii. a functional homologous gene of *PMT1* gene, which gene is capable of restoring parental O-mannosylation level by functional complementation when introduced into a *T. reesei* strain having a disruption in said *PMT1* gene, or,
iii. a polynucleotide encoding a polypeptide having at least 50%, at least 60%, at least 70%, at least 90%, or at least 95% identity with SEQ ID NO:2, said polypeptide having protein O-mannosyltransferase activity.

The disclosure also provides a protein or antibody composition obtainable by the method described herein.

DESCRIPTION OF THE FIGURES

Figure 1 depicts results for Southern analyses of *Trichoderma reesei pmt1* deletion strains expressing antibody MAB01. A) A 5.7 kb signal is expected from parental strains M124 and M304 with *pmt1* ORF probe after *Spe*I + *Xba*I digestion. No signal is expected from pure *pmt1* deletion strains. B) A 3.5 kb signal is expected for *pmt1* 5'flank probe from deletion strains after *Spe*I + *Asc*I digestion. C) A 1.7 kb signal is expected for *pmt1* 3'flank probe from deletion strains after *Asc*I + *Xba*I digestions. *Asc*I does not cut intact *pmt1* locus in close distance, therefore signals of over 16 kb (B) and 10 kb (C) are expected from parental strains M124 or M304. A 4.1 kb signal is expected from *Pme*I digested plasmid pTTv185 used as a control in hybridisations with both flank probes (B, C).

Figure 2 depicts a spectra of light chain of flask cultured parental *T. reesei* strain M317 (pyr4$^-$ of M304) (A) and Δpmt1 disruptant clone 26-8A (B), day 7.

CLAIMS

1. A PMT-deficient filamentous fungal cell comprising:
    a) a first mutation that reduces or eliminates an endogenous protease activity compared to a parental filamentous fungal cell which does not have said first mutation, said endogenous proteases being selected among aspartic proteases, trypsin-like serine proteases, subtilisin proteases, glutamic proteases and sedolisin proteases, and
    b) a second mutation in a protein O-mannosyltransferase (*PMT*) gene that reduces endogenous O-mannosyltransferase activity compared to a parental filamentous fungal cell which does not have said second mutation,

    wherein said filamentous fungal cell is selected from the group consisting of *Trichoderma*, *Neurospora*, *Myceliophthora* and *Chrysosporium* cell.

2. The PMT-deficient filamentous fungal cell of claim 1, wherein said second mutation that reduces the endogenous O-mannosyltransferase activity is a deletion or a disruption of a *PMT* gene encoding an endogenous protein O-mannosyltransferase activity.

3. The PMT-deficient filamentous fungal cell of claim 1 or 2, wherein said second mutation in a PMT gene is a mutation in either :
    a) *PMT1* gene comprising the polynucleotide of SEQ ID NO:1,
    b) a functional homologous gene of *PMT1* gene, which functional homologous gene is capable of restoring parental O-mannosylation level by functional complementation when introduced into a *T. reesei* strain having a disruption in said *PMT1* gene, or;
    c) a polynucleotide encoding a polypeptide having at least 50% identity with SEQ ID NO:2, said polypeptide having O-mannosyltransferase activity.

4. The PMT-deficient filamentous fungal cell of any one of claims 1-3, wherein said cell has a third mutation that reduces or eliminates the level of expression of an *ALG3* gene compared to the level of expression in a parental cell which does not have such third mutation.

5. The PMT-deficient filamentous fungal cell of Claim 4, further comprising a first polynucleotide encoding a N-acetylglucosaminyltransferase I catalytic domain and a

i. a mutation that reduces endogenous protein O-mannosyltransferase activity as compared to parental strain which does not have such mutation, and,

ii. a polynucleotide encoding a light chain antibody and a polynucleotide encoding a heavy chain antibody,

b) culturing the cell to produce said antibody, consisting of heavy and light chains, having reduced O-mannosylation,

wherein said filamentous fungal cell is selected from the group consisting of *Trichoderma*, *Neurospora*, *Myceliophthora* and *Chrysosporium* cell.