established as mammalian cell culture and therefore suffer from drawbacks when expressing mammalian proteins.

[0004] Thus, a need remains in the art for improved filamentous fungal cells, such as Trichoderma fungus cells, that can stably produce heterologous proteins, such as immunoglobulins, preferably at high levels of expression.

SUMMARY

[0005] Described herein are compositions including filamentous fungal cells, such as Trichoderma fungal cells having reduced or no detectable activity of at least three proteases, and having a recombinant polynucleotide encoding a heterologous polypeptide that is produced at increased levels. Further described herein are methods of improving heterologous polypeptide stability and methods of making heterologous polypeptides in which the proteases do not have the reduced activity..

[0005a] One aspect of the invention relates to a Trichoderma cell comprising at least three endogenous proteases having reduced or eliminated activity, and a recombinant polynucleotide encoding a heterologous polypeptide, wherein the polypeptide is produced at a level of at least two-fold higher than the production level of the polypeptide in a corresponding parental Trichoderma cell in which the proteases do not have the reduced or eliminated activity and wherein the Trichoderma cell has reduced or eliminated activity in at least gap1 protease, gap2 protease and pep3 protease.

[0005b] Another aspect relates to a method of improving stability of a polypeptide, comprising providing the Trichoderma cell as disclosed herein, and culturing the cell such that the heterologous polypeptide is expressed, wherein the heterologous polypeptide exhibits increased stability compared to the heterologous polypeptide produced in a corresponding parental Trichoderma cell in which the proteases do not have reduced activity,

[0005c] Still another aspect relates to a method of making a heterologous polypeptide, comprising providing the Trichoderma cell as disclosed herein, culturing the cell such that the heterologous polypeptide is expressed, and purifying the heterologous polypeptide.

[0006] Thus one aspect includes filamentous fungal cells having reduced or no detectable activity of at least three proteases, where the cell further contains a recombinant polynucleotide encoding a heterologous polypeptide produced at a level of at least 2-fold higher than the production level of the polypeptide in a corresponding parental filamentous fungal cell in which the proteases do not have the reduced activity. In certain embodiments, when the cell is an Aspergillus cell, the total protease activity is reduced to 50% or less of the total protease activity of the corresponding parental Aspergillus cell in which the protease do not have reduced activity. In other embodiments, the total protease activity of the filamentous fungal cell is reduced to 49% or less, 31% or less, of the total protease activity of the corresponding parental filamentous fungal cell in which the proteases do not have the reduced activity.

[0007] In certain embodiments, the expression level of the at least three proteases is reduced or eliminated. In certain embodiments, genes encoding the three proteases each comprise a mutation that reduces or eliminates the corresponding protease activity. In certain embodiments that may be combined with the preceding embodiments, the three protease encoding genes are

CLAIMS

We claim:

1. A filamentous fungal cell comprising at least three endogenous proteases having reduced activity, and a recombinant polynucleotide encoding a heterologous polypeptide, wherein the polypeptide is produced at a level of at least two-fold higher than the production level of the polypeptide in a corresponding parental filamentous fungal cell in which the proteases do not have the reduced activity.

2. The filamentous fungal cell of Claim 1, with the proviso that, when the cell is an Aspergillus cell, the total protease activity is reduced to 50% or less of the total protease activity of the corresponding parental Aspergillus cell in which the proteases do not have reduced activity.

3. The filamentous fungal cell of claim 1 or 2, wherein the total protease activity is reduced to 49% or less, preferably 31% or less, of the total protease activity of the corresponding parental filamentous fungal cell in which the proteases do not have the reduced activity.

4. The filamentous fungal cell of any one of claims 1-3, wherein at least three genes encoding endogenous proteases each comprise a mutation that reduces or eliminates the corresponding protease activity.

5. The filamentous fungal cell of any one of claims 1-4, wherein at least four genes encoding endogenous proteases each comprise a mutation that reduces or eliminates the corresponding protease activity.

6. The filamentous fungal cell of any preceding claim, wherein the fungal cell is a Trichoderma fungal cell, a Myceliophthora fungal cell, an Aspergillus fungal cell, a Neurospora fungal cell, a Penicillium cell, or a Chrysosporium fungal cell.

7. The filamentous fungal cell of any preceding claim, wherein the fungal cell is *Trichoderma reesei*.

8. The filamentous fungal cell of Claim 6 or 7, wherein said cell is wild type for pep4 protease.

9. The filamentous fungal cell of claim 6, 7 or 8, wherein the cell has reduced or no protease activity in at least the following three proteases: either pep1, tsp1 and slp1 or gap1, slp1, and pep1.

10. The filamentous fungal cell of claims 6 or 7, wherein the cell has reduced or no detectable protease activity in at least three or four proteases selected from the group consisting of pep1, pep2, pep3, pep4, pep5, pep8, pep11, pep12, tsp1, slp1, slp2, slp3, slp7, gap1, and gap2.

11. The filamentous fungal cell of claim 10, wherein each of the genes encoding the three or four proteases with reduced or no detectable protease activity comprises a mutation that reduces or eliminates the corresponding protease activity.

12. The filamentous fungal cell of claim 6 or 7, wherein the cell has reduced or no protease activity in at least eight proteases, each of the genes encoding the eight proteases comprises a mutation that reduces or eliminates the corresponding protease activity, and the eight proteases are pep1, tsp1, slp1, gap1, gap2, pep4, pep3, and pep5.

13. The filamentous fungal cell of claim 6 or 7, wherein the cell comprises three to six proteases having reduced or no detectable activity, and each of the three to six proteases are selected from the group consisting of pep1, pep2, pep3, pep4, pep5, tsp1, slp1, slp2, slp3, gap1, and gap2.

14. The filamentous fungal cell of any one of Claims 6-13, wherein the cell comprises a gene encoding an additional protease which comprises a mutation that reduces the corresponding protease activity, and the additional protease is selected from the group consisting of pep7, pep8, pep11, pep12, tpp1, gap2, slp3, slp5, slp6, slp7, and slp8.

15. The filamentous fungal cell of any preceding claim, wherein the heterologous polypeptide is a mammalian polypeptide.

16. The filamentous fungal cell of claim 15, wherein the mammalian polypeptide is glycosylated.

17. The filamentous fungal cell of claim 15, wherein the mammalian polypeptide is selected from the group consisting of an antibody and their antigen-binding fragments, a growth factor, an interferon, a cytokine, and an interleukin.

18. The filamentous fungal cell of claim 15, wherein the mammalian polypeptide is selected from the group consisting of insulin-like growth factor 1 (IGF1), human growth hormone (hGH), and interferon alpha 2b (IFNα2b).

19. The filamentous fungal cell of any preceding claim, wherein the fungal cell further comprises ALG3 having reduced activity.

20. The filamentous fungal cell of claim 19, wherein the gene encoding ALG3 comprises a mutation that reduces or eliminates the corresponding activity.

21. The filamentous fungal cell of any one of claims 1-20, wherein the fungal cell further comprises a polynucleotide encoding an α-1,2-mannosidase.

22. The filamentous fungal cell of any preceding claim, further comprising

an N-acetylglucosaminyltransferase I catalytic domain, and, optionally,

an N-acetylglucosaminyltransferase II catalytic domain.

23. The filamentous fungal cell of claim 22, further comprising a first polynucleotide encoding the N-acetylglucosaminyltransferase I catalytic domain and, optionally, a second polynucleotide encoding the N-acetylglucosaminyltransferase II catalytic domain.

24. The filamentous fungal cell of any preceding claim, further comprising a polynucleotide encoding a mannosidase II and/or a galactosyl transferase.

25. A method of improving stability of a polypeptide, comprising

a) providing the filamentous fungal cell of any preceding claim, and

b) culturing the cell such that the heterologous polypeptide is expressed,

wherein the heterologous polypeptide exhibits increased stability compared to the heterologous polypeptide produced in a corresponding parental filamentous fungal cell in which the proteases do not have reduced activity.

26. A method of making a heterologous polypeptide, comprising

a) providing the filamentous fungal cell of any of claims 1-24,

b) culturing the cell such that the heterologous polypeptide is expressed, and

c) purifying the heterologous polypeptide.

27. The method of claim 25 or claim 26, wherein the filamentous fungal cell further comprises a carrier protein.

28. The method of claim 27, wherein the carrier protein is CBH1.

29. The method of any one of claims 26-28 wherein the culturing is in a medium comprising a protease inhibitor.

30. The method of any one of claims 26-29 wherein the culturing is in a medium comprising one or two protease inhibitors selected from SBT1 and chymostatin.

CLAIMS

1. A Trichoderma cell comprising at least three endogenous proteases having reduced or eliminated activity, and a recombinant polynucleotide encoding a heterologous polypeptide, wherein the polypeptide is produced at a level of at least two-fold higher than the production level of the polypeptide in a corresponding parental Trichoderma cell in which the proteases do not have the reduced or eliminated activity and wherein the Trichoderma cell has reduced or eliminated activity in at least gap1 protease, gap2 protease and pep3 protease.

2. The Trichoderma cell of claim 1, wherein at least three genes encoding endogenous proteases each comprise a mutation that reduces or eliminates the corresponding protease activity.

3. The Trichoderma cell of claims 1 or 2, wherein the cell has reduced or no detectable protease activity in at least a further fourthprotease selected from the group consisting of pep1, pep2, pep4, pep5, pep8, pep11, pep12, tsp1, slp1, slp2, slp3, and slp7.

4. The Trichoderma cell of any preceding claim, wherein the heterologous polypeptide is a mammalian polypeptide.

5. The Trichoderma cell of claim 4, wherein the mammalian polypeptide is glycosylated.

6. The Trichoderma cell of claim 4, wherein the mammalian polypeptide is selected from the group consisting of an antibody and their antigen-binding fragments, a growth factor, an interferon, a cytokine, and an interleukin.

7. The Trichoderma cell of claim 4, wherein the mammalian polypeptide is selected from the group consisting of insulin-like growth factor 1 (IGF1), human growth hormone (hGH), and interferon alpha 2b (IFNα2b).

8. The Trichoderma cell of any preceding claim, wherein the Trichoderma cell further comprises ALG3 (Dolichyl-P-Man:Man(5)GlcNAc(2)-PP-dolichyl mannosyltransferase) having reduced activity.

9. The Trichoderma cell of claim 8, wherein the gene encoding ALG3 comprises a mutation that reduces or eliminates the corresponding activity.

10. The Trichoderma cell of any one of claims 1-9, wherein the Trichoderma cell further comprises a polynucleotide encoding an α-1,2-mannosidase.

11. The Trichoderma cell of any preceding claim, further comprising

an N-acetylglucosaminyltransferase I catalytic domain, and, optionally,

an N-acetylglucosaminyltransferase II catalytic domain.

12. The Trichodermacell of any preceding claim, further comprising a polynucleotide encoding a mannosidase II and/or a galactosyl transferase.

13. A method of improving stability of a polypeptide, comprising

a) providing the Trichoderma cell of any preceding claim, and

b) culturing the cell such that the heterologous polypeptide is expressed,

wherein the heterologous polypeptide exhibits increased stability compared to the heterologous polypeptide produced in a corresponding parental Trichoderma cell in which the proteases do not have reduced activity.

14. A method of making a heterologous polypeptide, comprising

a) providing the Trichoderma cell of any of claims 1-12,

b) culturing the cell such that the heterologous polypeptide is expressed, and

c) purifying the heterologous polypeptide.