misincorporation is thought to be caused by promiscuity of aminoacyl-tRNA synthetase (aaRS) for structurally related amino acids in the course of endogen substrate limitation (Feeney et al. 2013; Jakubowski, 2001). Misincorporation of free *meta*-tyrosine into cellular proteins as a potential cytotoxic mechanism for oxidized amino acids is reported in Gurer-Urhan et. al., (2006).

Summary

It has been found that supplementation of a culture medium with *meta*-tyrosine provides for an increased specific productivity (qP) of a eukaryotic (host) cell which produces a (non-endogenous) polypeptide. It has been found that the addition of *meta*-tyrosine to a cultivation of eukaryotic cells does not lead to a significant decrease in cell viability or final product titer. Cell growth in such a cultivation is reduced/affected in a negative manner (represented by reduced viable cell density (VCD) and overall biomass production (indicated as CTI)). In the current method it is not necessary to perform a temperature-, osmolality- or pH shift to increase the specific productivity of the cultivated cells. It is also not necessary to modulate the specific productivity by addition of drugs like valproic acid or sodium butyrate. The occurrence of amino acid sequence variants (SVs) due to alternative amino acid misincorporation during mRNA translation in the presence of *meta*-tyrosine is controlled by additional feeding of phenylalanine in non-limiting concentrations.

As reported herein is the use of *meta*-tyrosine for increasing the specific productivity of a eukaryotic host cell that produces/expresses/secretes a (non-endogenous/exogenous) polypeptide.

In one embodiment the eukaryotic host cell is a mammalian cell. In one embodiment the eukaryotic host cell is a Chinese Hamster Ovary (CHO) cell. In one embodiment the CHO cell is an in suspension growing CHO cell/cell line (= a CHO suspension cell/cell line). In one embodiment the CHO cell is a CHO-K1 cell.

One aspect as reported herein is the use of *meta*-tyrosine for increasing the specific productivity of a Chinese Hamster Ovary (CHO) cell that produces an exogenous polypeptide.

In one embodiment the *meta*-tyrosine is added to result at a (final) concentration of from 0.2 mM to 0.7 mM. In one embodiment the *meta*-tyrosine is added to result at a (final) concentration of from 0.25 mM to 0.6 mM. In one embodiment the *meta*-tyrosine is added to result at a (final) concentration of from 0.3 mM to 0.5 mM. In one embodiment

the *meta*-tyrosine is added to result at a (final) concentration of from 0.3 mM to 0.4 mM.

In one embodiment the specific productivity is increased by at least 5% compared to an identical production process without supplementation of *meta*-tyrosine. In one embodiment the specific productivity is increased by at least 10% compared to an identical production process without supplementation of *meta*-tyrosine. In one embodiment the specific productivity is increased by at least 20% compared to an identical production process without supplementation of *meta*-tyrosine. In one embodiment the specific productivity is increased by at least 25% compared to an identical production process without supplementation of *meta*-tyrosine.

In one embodiment the use is in a protein-free culture medium. In one embodiment the use is in a chemically defined culture medium. In one embodiment the use is in a protein-free, chemically defined culture medium.

In one embodiment the use is in a culture medium that additionally comprises phenylalanine in a non-limiting concentration. In one embodiment the use is in a culture medium that additionally comprises phenylalanine in a non-limiting concentration, wherein phenylalanine is added by continuous feeding or by one or more individual bolus shots of Phe stock solutions at the beginning or during the fermentation process.

In one embodiment the molar ratio of/for *meta*-tyrosine/phenylalanine is lower than or equal to 1.25. In one embodiment the final protein sequence fidelity with respect to Phe → m-Tyr misincorporation is higher than or equal to 95.0%. In one embodiment the molar ratio of/for *meta*-tyrosine/phenylalanine is lower than or equal to 1.25 and the final protein sequence fidelity with respect to Phe → m-Tyr misincorporation is higher than or equal to 95.0%. This means that 5% or less *meta*-tyrosine residues are misincorporated over correctly incorporated phenylalanine residues into the protein sequence. In one embodiment the molar ratio of/for *meta*-tyrosine/phenylalanine is lower than or equal to 0.25. In one embodiment the final protein sequence fidelity with respect to Phe → m-Tyr misincorporation is higher than or equal to 99.0%. In one embodiment the molar ratio of/for *meta*-tyrosine/phenylalanine is

lower than or equal to 0.25 and the final protein sequence fidelity with respect to Phe → m-Tyr misincorporation is higher than or equal to 99.0%. In one embodiment the molar ratio of/for *meta*-tyrosine/phenylalanine is lower than or equal to 0.125. In one embodiment the final protein sequence fidelity with respect to Phe → m-Tyr misincorporation is higher than or equal to 99.5%. In one embodiment the molar ratio of/for *meta*-tyrosine/phenylalanine is lower than or equal to 0.125 and the final protein sequence fidelity with respect to Phe → m-Tyr misincorporation is higher than or equal to 99.5%. In one embodiment the molar ratio of/for *meta*-tyrosine/phenylalanine is lower than or equal to 0.025. In one embodiment the final protein sequence fidelity with respect to Phe → m-Tyr misincorporation is higher than or equal to 99.9%. In one embodiment the molar ratio of/for *meta*-tyrosine/phenylalanine is lower than or equal to 0.025 and the final protein sequence fidelity with respect to Phe → m-Tyr misincorporation is higher than or equal to 99.9%.

In one embodiment the use is at a constant temperature.

In one embodiment the use is at a temperature which is reduced in the course of the use.

In one embodiment the use is at a constant pH.

In one embodiment the polypeptide is an immunoglobulin or a variant thereof or a fragment thereof or a fusion thereof. In one embodiment the polypeptide is a human or humanized immunoglobulin or a variant thereof or a fragment thereof or a fusion thereof. In one embodiment the polypeptide is a humanized antibody. In one embodiment the polypeptide is a humanized monoclonal antibody.

One aspect as reported herein is a process for producing a polypeptide in a eukaryotic host cell expressing a nucleic acid encoding the polypeptide, comprising culturing the eukaryotic host cell in a culture medium comprising *meta*-tyrosine.

In one embodiment the *meta*-tyrosine is added to result at a (final) concentration of from 0.2 mM to 0.7 mM. In one embodiment the *meta*-tyrosine is added to result at a (final) concentration of from 0.25 mM to 0.6 mM. In one embodiment the *meta*-tyrosine is added to result at a (final) concentration of from 0.3 mM to 0.5 mM. In one embodiment

the *meta*-tyrosine is added to result at a (final) concentration of from 0.3 mM to 0.4 mM.

In one embodiment the specific productivity is increased by at least 5% compared to an identical production process without supplementation of *meta*-tyrosine. In one embodiment the specific productivity is increased by at least 10% compared to an identical production process without supplementation of *meta*-tyrosine. In one embodiment the specific productivity is increased by at least 20% compared to an identical production process without supplementation of *meta*-tyrosine. In one embodiment the specific productivity is increased by at least 25% compared to an identical production process without supplementation of *meta*-tyrosine.

In one embodiment the eukaryotic host cell is a mammalian cell. In one embodiment the eukaryotic host cell is a Chinese Hamster Ovary (CHO) cell. In one embodiment the CHO cell is a CHO suspension cell/a CHO cell growing in suspension. In one embodiment the CHO cell is a CHO-K1 cell.

In one embodiment the process is performed in a protein-free culture medium. In one embodiment the process is performed in a chemically defined culture medium. In one embodiment the process is performed in a protein-free, chemically defined culture medium.

In one embodiment the process is performed in a culture medium that additionally comprises phenylalanine in a non-limiting concentration. In one embodiment the process is performed in a culture medium that additionally comprises phenylalanine in a non-limiting concentration, wherein phenylalanine is added by continuous feeding or by one or more individual bolus shots of Phe stock solutions at the beginning or during the fermentation process.

In one embodiment the molar ratio of/for *meta*-tyrosine/phenylalanine is lower than or equal to 1.25. In one embodiment the final protein sequence fidelity with respect to Phe → m-Tyr misincorporation is higher than or equal to 95.0%. In one embodiment the molar ratio of/for *meta*-tyrosine/phenylalanine is lower than or equal to 1.25 and the final protein sequence fidelity with respect to Phe → m-Tyr misincorporation is higher than or equal to 95.0%. This means that 5% or less

meta-tyrosine residues are misincorporated over correctly incorporated phenylalanine residues into the protein sequence.

In one embodiment the molar ratio of/for *meta*-tyrosine/phenylalanine is lower than or equal to 0.25. In one embodiment the final protein sequence fidelity with respect to Phe → m-Tyr misincorporation is higher than or equal to 99.0%. In one embodiment the molar ratio of/for *meta*-tyrosine/phenylalanine is lower than or equal to 0.25 and the final protein sequence fidelity with respect to Phe → m-Tyr misincorporation is higher than or equal to 99.0%.

In one embodiment the molar ratio of/for *meta*-tyrosine/phenylalanine is lower than or equal to 0.125. In one embodiment the final protein sequence fidelity with respect to Phe → m-Tyr misincorporation is higher than or equal to 99.5%. In one embodiment the molar ratio of/for *meta*-tyrosine/phenylalanine is lower than or equal to 0.125 and the final protein sequence fidelity with respect to Phe → m-Tyr misincorporation is higher than or equal to 99.5%.

In one embodiment the molar ratio of/for *meta*-tyrosine/phenylalanine is lower than or equal to 0.025. In one embodiment the final protein sequence fidelity with respect to Phe → m-Tyr misincorporation is higher than or equal to 99.9%. In one embodiment the molar ratio of/for *meta*-tyrosine/phenylalanine is lower than or equal to 0.025 and the final protein sequence fidelity with respect to Phe → m-Tyr misincorporation is higher than or equal to 99.9%.

In one embodiment the process is performed at a constant temperature. In one embodiment the process is performed at a temperature which is reduced in the course of the use.

In one embodiment the process is performed at a constant pH.

In one embodiment the polypeptide is an immunoglobulin or a variant thereof or a fragment thereof or a fusion thereof. In one embodiment the polypeptide is a human or humanized immunoglobulin or a variant thereof or a fragment thereof or a fusion thereof. In one embodiment the polypeptide is a humanized antibody. In one embodiment the polypeptide is a humanized monoclonal antibody.

One aspect as reported herein is a culture medium comprising *meta*-tyrosine and phenylalanine in a molar ratio which is lower than or equal to 1.25. As reported herein is a culture medium comprising *meta*-tyrosine and phenylalanine in a molar ratio which is lower than or equal to 1.25 and the final protein sequence fidelity with respect to Phe → m-Tyr misincorporation is higher than or equal to 95.0%. This means that 5% or less *meta*-tyrosine residues are misincorporated over correctly incorporated phenylalanine residues into the protein sequence.

As reported herein is a culture medium comprising *meta*-tyrosine and phenylalanine in a molar ratio which is lower than or equal to 0.25. As reported herein is a culture medium comprising *meta*-tyrosine and phenylalanine in a molar ratio which is lower than or equal to 0.25 and the final protein sequence fidelity with respect to Phe → m-Tyr misincorporation is higher than or equal to 99.0%.

As reported herein is a culture medium comprising *meta*-tyrosine and phenylalanine in a molar ratio which is lower than or equal to 0.125. As reported herein is a culture medium comprising *meta*-tyrosine and phenylalanine in a molar ratio which is lower than or equal to 0.125 and the final protein sequence fidelity with respect to Phe → m-Tyr misincorporation is higher than or equal to 99.5%.

As reported herein is a culture medium comprising *meta*-tyrosine and phenylalanine in a molar ratio which is lower than or equal to 0.025. As reported herein is a culture medium comprising *meta*-tyrosine and phenylalanine in a molar ratio which is lower than or equal to 0.025 and the final protein sequence fidelity with respect to Phe → m-Tyr misincorporation is higher than or equal to 99.9%.

In one embodiment the culture medium is a protein-free culture medium. In one embodiment the culture medium is a chemically defined culture medium. In one embodiment the culture medium is a protein-free, chemically defined culture medium.

One aspect as reported herein is a method for increasing the specific productivity of a eukaryotic host cell, wherein the culture medium comprises *meta*-tyrosine.

Detailed Description of the Invention

Modulation of the specific productivity (qP) of a eukaryotic cell is thought to be a powerful tool to address alterations in product quality attributes such as micro heterogeneity in protein N-glycosylation, charge variants, protein aggregates and

14. Culture medium comprising *meta*-tyrosine and phenylalanine in a molar ratio which is lower than or equal to 1.25.

15. Culture medium according to claim 14, wherein the culture medium is a chemically defined culture medium.

16. A method for increasing the specific productivity of a eukaryotic host cell, wherein the culture medium comprises *meta*-tyrosine.