

Claims

1. A fungal host cell comprising an expression cassette comprising a nucleotide sequence coding for an animal-derived food-protein of interest, which nucleotide sequence is operably
5 linked to at least one regulatory sequence that is capable of effecting expression of the encoded protein of interest by the fungal host cell.
2. The fungal host cell according to claim 1, wherein the regulatory sequence is the promoter of a highly expressed fungal protein, wherein preferably the highly expressed fungal protein is
10 selected from acid α -amylase, α -amylase, TAKA-amylase, glucoamylase, xylanase, cellobiohydrolase, pyruvate kinase, glyceraldehyde-phosphate dehydrogenase, alcohol dehydrogenase, aldehyde dehydrogenase, sucrase, acetamidase, superoxide dismutase, more preferably wherein the promoter is an *A. niger* glucoamylase promoter.
- 15 3. The fungal host cell according to claim 1 or claim 2, wherein the nucleotide sequence coding for the animal-derived food-protein of interest is codon optimized with reference to the native codon usage of the highly expressed fungal protein from which the promoter is derived, wherein preferably the highly expressed fungal protein is an *A. niger* glucoamylase.
- 20 4. The fungal host cell according to any one of claims 1 – 3, wherein the expression cassette is integrated in a locus of a gene coding for a highly expressed fungal protein and wherein preferably, the locus is an amplified locus and wherein more preferably, the locus is an *A. niger glaA* locus.
- 25 5. The fungal host cell according to any one of claims 1 – 4, wherein the expression cassette comprises a nucleotide sequence encoding a signal sequence from a highly expressed secreted fungal protein, and optionally a pro-sequence, operably linked in frame to the nucleotide sequence coding for the animal-derived food-protein of interest, wherein preferably the signal sequence and optional a pro-sequence are the *A. niger* glucoamylase
30 prepro-sequences.
6. The fungal host cell according to claim 5, wherein the expression cassette encodes a fusion protein comprising, in a N- to C-terminal direction: an *A. niger* glucoamylase pre-pro sequence; optionally, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100% of the mature *A. niger*
35 glucoamylase amino acid sequence; optionally, a cleavable linker polypeptide; fused to the N-terminus of the protein of interest.
7. The fungal host cell according to any one of claims 1 – 6, wherein the host cell is a yeast or a filamentous fungus, preferably, a filamentous fungal host cell belong to a species selected
40 from *Alternaria alternata*, *Apophysomyces variabilis*, *Aspergillus* spp., *Aspergillus fumigatus*,

- Aspergillus flavus*, *Aspergillus oryzae*, *Aspergillus niger*, *Aspergillus awamori*, *Aspergillus nidulans*, *Aspergillus terreus*, *Cladosphialophora* spp., *Fonsecaea pedrosoi*, *Fusarium* spp., *Fusarium oxysporum*, *Fusarium solani*, *Lichtheimia* spp., *Lichtheimia corymbifera*, *Lichtheimia ramosa*, *Myceliophthora* spp., *Myceliophthora thermophila*, *Rhizopus* spp., *Rhizopus microsporus*, *Rhizomucor* spp., *Rhizomucor pusillus*, *Rhizomucor miehei*, *Trichoderma* spp., *Trichoderma reesei*, *Trichophyton* spp., *Trichophyton interdigitale*, and *Trichophyton rubrum*, of which a strain of the species *Aspergillus niger* is most preferred.
8. The fungal host cell according to claim 7, wherein the host cell is a strain of a filamentous fungus that has the ability to grow in a yeast-like morphology, wherein preferably the strain is *Aspergillus niger* strain CICC2462, or a strain that is a single colony isolate and/or a derivative of strain CICC2462.
9. The fungal host cell according to any one of claims 1 – 8, wherein animal-derived food-protein of interest is a hemeprotein, a milk protein or an egg protein, preferably an egg white protein and most preferably an ovalbumin.
10. The fungal host cell according to claim 10, wherein the ovalbumin comprises an amino acid sequence with at least 50% identity to the amino acid sequence of an ovalbumin from a bird selected from the group consisting of chicken, pelican, quail, pigeon, ostrich, plover, turkey, duck, goose, gull, guineafowl, junglefowl, peafowl, partridge, pheasant, emu, rhea and kiwi.
11. A process for producing an animal-derived food-protein of interest, the process comprising the steps of:
- a) culturing the fungal host cell as defined in any one of claims 1 to 10 in a medium in a fermenter under condition conducive to the expression of the protein of interest; and,
- b) optionally, recovery of the protein of interest,
- wherein, preferably, the protein of interest is an ovalbumin.
12. The process according to claim 11, wherein in step a) the fungal host cell is cultured at a pH that is equal to or higher than pH 5.0, 5.5, 6.0, 6.5, 7.0 or 7.5, or that is equal to or lower than pH 9.0, 8.5 or 8.0.
13. The process according to claims 11 or 12, wherein in step a) the input of mechanical power into the medium in the fermenter is no more than 2.5, 2.0, 1.8, 1.6, 1.4, 1.0, 0.5, 0.2 or 0.1 kW/m³.
14. The process according to any one of claims 11-13, wherein in step b) the recovery of the protein of interest comprises at least a first anion exchange step wherein the culture medium obtained in step a) is applied to an anion exchange column at an ionic strength that is less

- than the equivalent of 0.08 M NaCl at a pH in the range of pH 6 to 9 and wherein the protein of interest is collected in the flow-through, wherein, optionally the flow-through obtained in the first anion exchange step is subjected to a second anion exchange step under identical conditions as the first step and wherein the protein of interest is collected in the flow-through from the second anion exchange step, and wherein, preferably, the protein of interest is an ovalbumin.
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15. A process for purifying an animal-derived food-protein of interest from a spent culture medium of a fungal host cell wherein the protein has been expressed, the process comprising the steps of:
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- a) a first anion exchange step wherein the spent culture medium is applied to an anion exchange column at an ionic strength that is less than the equivalent of 0.08 M NaCl at a pH in the range of pH 6 to 9 and wherein the protein of interest is collected in the flow-through, wherein preferably, the protein of interest is an ovalbumin; and,
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- b) optionally, a second anion exchange step wherein the flow-through obtained in step a) is subjected to anion exchange under identical conditions as the first step, and wherein the protein of interest is collected in the flow-through, and wherein preferably, the protein of interest is an ovalbumin.
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16. Use of the fungal host cell according to any one of claims 1 to 10 or a process according to any one of claims 11 to 15, for the production of an animal-derived food-protein of interest, wherein, preferably, the protein of interest is an ovalbumin.