[Non-Patent Document 15] Yurimoto H, Komeda T, Lim CR, Nakagawa T, Kato N, Sakai Y, Regulation and evaluation of five methanol-inducible promoters in methylotrophic yeast Candida boidinii. Biochim. Biophys. Acta. (2000) 1493(1-2): 56-63
[Non-Patent Document 16] Yurimoto H, Hasegawa T, Sakai Y, Kato N, Characterization and High-level production of D-amino acid oxidase in Candida boidinii. Biosci. Biotechnol. Biochem. (2001) 65(3), 627-633
[Non-Patent Document 17] Sakai Y, Yoshida H, Yurimoto H, Yoshida N, Fukuya H, Takabe K, Kato N, Production of fungal fructosyl amino acid oxidase useful for diabetic diagnosis in the peroxisome of Candida boidnii. FEBS Lett. (1999) 459, 233-237
[Non-Patent Document 18] Nishikawa M, Hagishita T, Yurimoto H, Kato N, Sakai Y, Hatanaka T, Primary structure and expression of peroxisomal acetylspermidine oxidase in the methylotrophic yeast Candida boidinii. FEBS Lett. (2000) 476, 150-154

Summary

Problems to be Solved by the Invention

[0012]

It is an object of the present invention to provide a production system that is capable of high-level secretory production of a protein (and in particular, a protein with a complicated structure, such as a structure with S-S bonds) in a host cell such as yeast, is suitable for industrial production with high safety, and does not require explosion-proof facilities.

Means for Solving the Problem

[0013]

The present inventors have conducted concentrated studies in order to attain the above objects. As a result, they discovered that a yeast strain into which a chaperone gene has been introduced and in which the aox1 gene encoding alcohol oxidase has been disrupted may be used, so that high-level secretory production of a target protein induced by low-concentration methanol would become possible under the control of the aox1 promoter. The present inventors also discovered that acidic proteases, such as proteinase B (PRB1) and proteinase A (PEP4), were significantly associated with degradation of the target protein expressed in yeast, and they confirmed that regulation of the pH level of a medium aimed at

disruption of the prb1 gene encoding proteinase B and/or suppression of activity of acidic protease such as proteinase A in yeast would lead to significant improvement in the secretory production amount of the target protein.

[0014]

The high-level protein secretory production system comprising the above described features in combination enables a significant reduction in the amount of methanol to be added. Thus, such system can be used as a highly safe production system that is suitable for industrial production. The present invention has been completed on the basis of such findings.

[0015]

Specifically, the present invention includes the following. In a first aspect, the present invention is directed to a transformed yeast into which a chaperone gene has been introduced and in which the aox1 gene has been disrupted and a protease gene has been disrupted. In another aspect, the present invention is directed to the use of the transformed yeast according to the present invention for the production of a target protein. In still another aspect, the present invention is directed to a method for producing a protein comprising culturing the transformed yeast according to the present invention in a medium and sampling a target protein from the culture product, wherein culture is conducted in a medium at a methanol concentration of 0.5 % (v/v) or less. In yet another aspect, the present invention is directed to a method for producing a transformed yeast comprising step (i) in addition to both step (ii) and (iii): (i) a step of introducing a chaperone gene into yeast; and (ii) a step of disrupting the aox1 gene in yeast; and (iii) a step of disrupting the prb1 gene in yeast.

Also described herein is the following:

(1) A transformed yeast into which a chaperone gene has been introduced and in which the aox1 gene has been disrupted.

(2) The transformed yeast according to (1), wherein the chaperone gene is at least one gene selected from the group consisting of genes (a) to (d) below:

(a) a gene encoding PDI1, ERO1, Kar2, MPD1, SCJ1, EUG1, or HSP104 derived from *Ogataea minuta* (*O. minuta*);

(b) a gene encoding PDI1, MPD1, SCJ1, ERO1, FKB2, JEM1, LHS1, MPD2, ERJ5, or EUG1 derived from *Saccharomyces cerevisiae* (*S. cerevisia*);

(c) a gene encoding PDI, ERO1-Lα, ERO1-Lβ, or GRP78 derived from a human; and

(d) a gene exhibiting 95% or higher sequence homology to a base sequence of any of the genes (a) to (c).

(3) The transformed yeast according to (1), wherein the chaperone gene is at least one gene selected from the group consisting of genes (a) to (g) below:

(a) a gene encoding PDI1 derived from *O. minuta*;

(b) a gene encoding ERO1 derived from *O. minuta*;

(c) a gene encoding Kar2 derived from *O. minuta*;

(d) a gene encoding PDI1 derived from *S. cerevisiae*;

(e) a gene encoding PDI derived from a human;

(f) a gene encoding ERO1 derived from a human; and

Claims

1. A transformed yeast into which a chaperone gene has been introduced and in which the aox1 gene has been disrupted and a protease gene has been disrupted.

2. The transformed yeast according to claim 1, wherein the chaperone gene is at least one gene selected from the group consisting of genes (a) to (d) below:

(a) a gene encoding PDI1, ERO1, Kar2, MPD1, SCJ1, EUG1, or HSP104 derived from *Ogataea minuta* (*O. minuta*);

(b) a gene encoding PDI1, MPD1, SCJ1, ERO1, FKB2, JEM1, LHS1, MPD2, ERJ5, or EUG1 derived from *Saccharomyces cerevisiae* (*S. cerevisia*);

(c) a gene encoding PDI, ERO1-Lα, ERO1-Lβ, or GRP78 derived from a human; and

(d) a gene exhibiting 95% or higher sequence homology to a base sequence of any of the genes (a) to (c).

3. The transformed yeast according to claim 1, wherein the chaperone gene is at least one gene selected from the group consisting of genes (a) to (g) below:

(a) a gene encoding PDI1 derived from *O. minuta*;

(b) a gene encoding ERO1 derived from *O. minuta*;

(c) a gene encoding Kar2 derived from *O. minuta*;

(d) a gene encoding PDI1 derived from *S. cerevisiae*;

(e) a gene encoding PDI derived from a human;

(f) a gene encoding ERO1 derived from a human; and

(g) a gene exhibiting 95% or higher sequence homology to a base sequence of any of the genes (a) to (f).

4. The transformed yeast according to claim 1, wherein the chaperone gene is any of the chaperone genes (a) to (g) below:

(a) a combination of a gene encoding PDI1, a gene encoding ERO1, and a gene encoding Kar2 derived from *O. minuta*;

(b) a combination of a gene encoding PDI1 and a gene encoding Kar2 derived from *O. minuta*;

(c) a combination of a gene encoding PDI derived from a human and a gene encoding ERO1 derived from *O. minuta*;

(d) a combination of a gene encoding PDI1 and a gene encoding ERO1 derived from *O. minuta*;

(e) a combination of a gene encoding PDI derived from a human, a gene encoding ERO1-Lβ derived from a human, and a gene encoding GRP78 derived from a human;

(f) a combination of a gene encoding PDI derived from a human, a gene encoding ERO1 derived from *O. minuta*, and a gene encoding GRP78 derived from a human; and

(g) a gene exhibiting 95% or higher sequence homology to a base sequence of any of the genes (a) to (f).

5. The transformed yeast according to claim 1, wherein the protease gene is a prb1 gene.

6. The transformed yeast according to any of claims 1 to 5, wherein the yeast is a methylotrophic yeast.

7. The transformed yeast according to any of claims 1 to 6, which comprises a gene encoding a target protein introduced thereinto.

8. Use of the transformed yeast according to any of claims 1 to 7 for the production of a target protein.

9. A method for producing a protein comprising culturing the transformed yeast according to claim 7 in a medium and sampling a target protein from the culture product, wherein culture is conducted in a medium at a methanol concentration of 0.5 % (v/v) or less.

10. The method for producing a protein according to claim 9, wherein culture is conducted under conditions in which protease activity is inhibited.

11. The method for producing a protein according to claim 9 or 10, wherein culture is conducted in a medium with a pH of 6.0 to 7.5.

12. The method for producing a protein according to any of claims 9 to 11, wherein a nitrogen source is added to the medium.

13. The method for producing a protein according to any of claims9 to 12, wherein methanol is not added to the medium.

14. A method for producing a transformed yeast comprising step (i) in addition to both step (ii) and (iii):

(i) a step of introducing a chaperone gene into yeast; and

(ii) a step of disrupting the aox1 gene in yeast; and

(iii) a step of disrupting the prb1 gene in yeast.

15. The method of production according to claim 14, which further comprises a step of introducing a gene encoding a target protein.