the control sample. Meanwhile, the Δaox1+PEK dprb1 strain exhibited a band intensity of 143819 with the addition of methanol at a lower concentration than that in the case of the NBRC10746+PEK strain and the NBRC10746+PEK dprb1 strain. That is, the Δaox1+PEK dprb1 strain exhibited improvement in secretory production that was about 1.8 times greater than that of the control sample. In particular, the Δaox1+PEK dprb1 strain exhibited high-level secretory productivity with the addition of methanol at a lower concentration than that in the case of the control sample.

[0090]

[Example 10] Comparison of KEX2 protein enzyme activity

Fig. 6-2 shows enzyme activity of KEX2 secreted and produced in Example 9. Enzyme activity was evaluated by fluorescence detection of AMC (7-amino-4-methylcoumarin) released upon the reaction of KEX2 with Boc-Leu-Arg-Arg-MCA (4-methylcoumaryl-7-amide) (#3140-v, Peptide Institute, Inc.) as a substrate. The strains were removed from the culture supernatant via centrifugation at 3,100 × g and 4°C for 5 minutes, and the resulting culture supernatant was designated as a KEX2-producing sample. After the sample had been adequately diluted with 100 mM Tris (pH 7.0), 100 μl of the diluted sample was mixed with 100 μl of the substrate solution (400 mM Tris (pH 7.0), 2 mM $CaCl_2$, 0.2% lubrol, 100 μM BOC-Leu-Arg-Arg-MCA), the mixture was subjected to reaction at 28°C for 30 minutes, and 50 μl of a reaction terminator (5 mM EGTA (Na)) was added to terminate the reaction. Thereafter, AMC was quantified using a fluorescence plate reader (excitation wavelength: 355 nm; measurement wavelength: 460 nm). The AMC standard sample (#3099-v, manufactured by Peptide Institute, Inc.) was diluted and subjected to detection in the same manner, so as to prepare a calibration curve, and the KEX2 protease activity in the sample was determined. A unit of KEX2 activity was defined as the KEX2 content that releases 1 pmol of AMC every minute under the reaction conditions described above. The Δaox1+PEK dprb1 strain exhibited improvement in enzyme activity that was ▪about 1.8 times greater than that of the control (the NBRC10746+PEK strain).

[0091]

[Example 11] Amount of HSA protein secretion induced by methanol at deep well plate scale

(1) Preparation of hsa expression plasmid

HSA (human serum albumin) (GenBank Accession Number NP000468) comprises an amino acid sequence of 609 amino acids (SEQ ID NO: 34). In order to express an α-factor pre-sequence and an α-factor pro-sequence derived from *S. cerevisiae* and HSA in the form of a fusion protein, DNA as shown in SEQ ID NO: 26 was artificially synthesized at Life technologies in accordance with the codon usage frequency of *P. pastoris*. A vector comprising the artificially synthesized DNA was digested with the restriction enzymes *Xba*I

16. The method for producing a protein according to any of claims 12 to 15, wherein the methanol is not added to the medium.

17. The method for producing a protein according to any of claims 12 to 15, wherein the amount of methanol added to the medium is 2% (v/v) or less.

18. A target protein produced by the method according to any of claims 12 to 17.

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

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

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

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

20. The method of production according to claim 19, which further comprises a step of introducing a gene encoding a target protein.