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Cell Surface Engineering in *Aspergillus oryzae*

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Research aims

Cell surface engineering is an innovative approach expressing heterologous proteins in the surface of microorganisms that are expected to offer functional protein-coated cells as novel biocatalysts. In vivo display of GFP, glucoamylase, antigens, and metal-adsorbing peptides were successfully achieved in bacteria and yeast by expression of fusion protein of functional peptides and cell surface anchor. However, cell surface expression system has not yet developed in filamentous fungi because of lack of anchors. Our main purpose is search for useful cell surface anchor protein in filamentous fungus *A. oryzae*, which has been widely used for Japanese traditional fermentative food production.

Methods

We isolated *nagA* and five *hyp* genes encoding β-N-acetylglucosaminidase and hydrophobins, respectively, which are considered to be localized to the cell surface of *A. oryzae*. The fusion genes of NagA-eGFP and HypA-eGFP were constructed and introduced into *A. oryzae*. Expression and localization of the fusion proteins were investigated by Western blot analysis and fluorescent microscope.

Results

β-N-acetylglucosaminidase has GlcNAc oligomer hydrolyzing activity and has shown to be involved in the formation of septa in filamentous fungi and germ tube in yeast. The *nagA* gene of *A. oryzae* encodes a protein of 600 amino acids with considerable homology to β-N-acetylglucosaminidases of various bacteria and fungi. We observed at least a part of cell surface localization of NagA-eGFP fusion on the cell surface of *A. oryzae* hyphae in the solid culture (data not shown). However, the cell surface absorbing-stability of the fusion protein was not high.

Hydrophobins have characteristic hydrophathy patterns, and contain eight conserved Cys residues. Cell surface of aerial hyphae, conidia, fruiting bodies and spores of filamentous fungi and mushrooms are covered with hydrophobin. Hydrophobins are secreted from fungus cells and self-assembled at the cell surface to form a hydrophobic layer. Based on the genome database of *A. oryzae,* we isolated possible hydrophobin encoding genes (*hypA–E*). The HypA from *A. oryzae* is highly homologous with the conidial hydrophobin RodA in *A. nidulans* (Fig. 1).

By western blot analysis, we found the solid-state culture specific expression of *hypA* and *hypB* mRNA. The transcriptional regulation of *hypA* and *hypB* genes suggests the aerial hyphae and conidia specific localization of hy-

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RodA    MKFSIAAAVVAFAASVAALPPAHDSQFAGNGVGNKGNSNVKFPVPENVTVKQASDKC--GDQAQLS-CC
HypA    MQFS-VAAVLALATAVAALP-PASGTGAGQQVGHSKN---DFPLPKELTTKQAADKC--GDQAQLT-CC
HypB    MKFFAVAALFATAAMAAPGS--APVPGAAAAAGNGNA-----PVINQTQQKAFTDACSAKGNHPV--CC
HypC    MKVTLLTTLCVLGVASSADP-------TAQ--------------------------C---DNGPVQ-CC
HypD    MHSTNIFNFFMLAVAAASAAT---ISKAGDSKALQKVAEGK---------------C---DIGNTA-CC
HypE    MKFLHTIALIATFTVASATP-------AGSTPSQ----------------------C---TAAQANKCC

RodA    NKATYAGDTTTVDEGLLSGALSGLIGAGSGAEGLGLFDQCSKLDVAVLIG---IQDLVNQK--CKQNIA
HypA    NKTVKTGDFTQVEEGLLAGLLSNLLGAGQGSQGLGLLDEC----TNIPVIPIISIASPQEK--CKQPIS
HypB    -DQIDTSKTTTVNEGLLGGLLGEGLGGVLNNLVGGEPGAC-----------SGLVSALNKQ--CQTSIG
HypC    ----ATGLPTDSVVSPLLGLLGVVVPDMSTPVGLT----C--------------NPIVQGGS-CPGHPV
HypD    -----NNVHEEKDERLFNLVKQGLIDILAGNEDYA----CAKSGVIDEWNLFSLVKQTNDGPVCKNVTA
HypE    ------------TGLTNGILNLNVLPAL----------C--------------LPLVGS---CNNQAA

RodA    CC-QNSPSD--ASGSLIG-IGLP-C-----VALGSIL--   157
HypA    CC-QNTKSS--ADGDLVG-IGLP-C-----IALGSLL--   151
HypB    CCQQNAKGDNYQSGLLNLNLQAP-C------LLSNGL--   146
HypC    CC---------TGNNFNGLLALG-C----TPVLST----    99
HypD    CC---------SSGK---------C-----VAIDG----   119
HypE    CC---------ETNGIVAPVELPHCGLMTRRLDMIYPI     96
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Fig. 1. Amino acid alignment of hydrophobins (HypA–E) from *A. oryzae* and *A. nidulans* RodA. Conserved Cys residues are underlined.

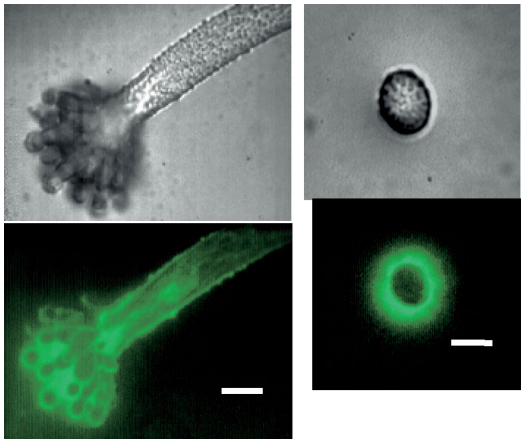

Fig. 2. HypA-eGFP is specifically localized at the surface of conidiophore and conidium of *A. oryzae*. Bar: 20 μm (left), 5 μm (right)

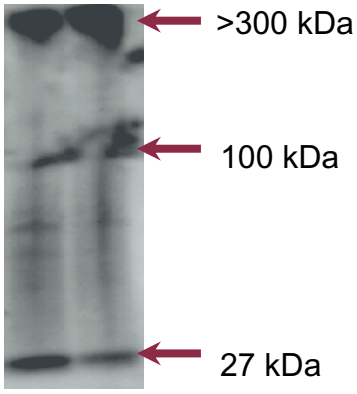

Fig. 3. HypA-eGFP is self-assembled on the surface of *A. oryzae*. Western blot analysis using anti-GFP antibody on the *A. oryzae* cells expressing the *hypA-egfp* gene on the solid-state medium.

drophobins. To investigate the localization of HypA protein, we constructed a plasmid carrying *hypA-egfp* fusion gene driven by the *hypA* promoter and introduced into *A. oryzae* niaD300.

The fluorescence in the hyphae of the transformant was hardly observed in submerged cultures. On the other hand, significant fluorescence was observed in the cell wall of aerial structures particularly the conidia (Fig. 2). eGFP (27 kDa) is relatively large tag for HypA (16 kDa). Western blot analysis on the *A. oryzae* transformant cells revealed three signals. The 27 kDa band is considered to be degraded product and the 100 kDa band may be HypA-eGFP oligomer. The most significant band with size more than 300 kDa was assumed to be the self-assembled HypA-eGFP (Fig. 3). Hydrophobins in general are predominantly present as assemblages that are completely insoluble in hot SDS solutions. Our observations indicated that eGFP-tagged HypA was successfully expressed and self-assembled specifically at the surface of aerial hyphae and conidia. We expect that HypA will be useful cell surface anchor for exhibiting the functional peptides on the surface of filamentous fungus *A. oryzae*.