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Transcriptional Analysis of Genes for Energy Catabolism and Hydrolytic Enzymes in the Filamentous Fungus *Aspergillus oryzae* Using cDNA Microarrays

Hiroshi MAEDA

Department of Molecular and Cell Biology, Graduate School of Agricultural Science, Tohoku University

Aspergillus oryzae is a fungus used extensively in the fermentation industry. We constructed cDNA microarrays comprising 2070 highly expressed cDNAs selected from the ~6000 nonredundant expressed sequence tags (ESTs) in the *A. oryzae* EST database. Using the cDNA microarrays, we analyzed the gene expression profiles of *A. oryzae* cells grown under the glucose-rich (AC) and glucose-depleted (AN) liquid culture conditions. In particular, transcription levels of most catabolic genes of the glycolytic pathway (EMP) and tricarboxylic acid cycle (TCA) were higher under AC than AN conditions, suggesting that *A. oryzae* uses both EMP and TCA for glucose metabolism under AC conditions. Therefore, the gene expression profiles for glucose metabolism in *A. oryzae* differ from those observed for *Saccharomyces cerevisiae* and *Trichoderma reesei*. We further studied the gene expression of hydrolytic enzymes and those of energy catabolism by using three industrial solid-phase biomass media. The wheat-bran culture gave the richest gene expression profile of the hydrolytic enzymes and the lowest expression levels of the catabolic genes among the three media. The low expression levels of catabolic genes in the wheat-bran culture may release catabolite repression, consequently leading to the rich expression profiles of the hydrolytic enzymes.

Methods

We used *Aspergillus oryzae* RIB40 (ATCC 42149), which was used for the *A. oryzae* EST project and the *A. oryzae* genome sequencing project. The mycelia were cultured in YPD (the glucose-rich "AC condition"). The mycelia in the YPD culture were washed and transferred to Czapek-Dox medium without a carbon source and were used after cultivation (the glucose-depleted "AN condition"). The solid-phase media, wheat-bran, rice bran, and soybean-curd refuse, then were inoculated with 5×10^6 conidia and incubated at 30°C. The set of 2070 amplified DNAs was used for preparation of cDNA microarrays. From separate mRNA samples, cDNA was prepared and labeled with the fluorochromes cyanine-3 (Cy3) and cyanine-5 (Cy5).

1. Glycolytic pathway (EMP)

Figure 1 shows the expression of selected glycolytic genes as determined by the cDNA microarray. The genes in the EMP were highly expressed in the presence of glucose (AC). In comparison, the expression of the genes for acetaldehyde dehydrogenase (*aldA*), acetyl-CoA synthase (*facA*), and alcohol dehydrogenase II (*adhB*) was significantly higher in the absence of glucose (AN) than in its presence (AC).

2. Tricarboxylic acid (TCA) cycle

We compared the expression levels of genes encoding enzymes of the TCA cycle under AC and AN conditions (Fig. 1). Except for that of the genes in TCA were upregulated under AC conditions. Whereas we found that the genes for succinate dehydrogenase and malate dehydrogenase were almost constitutively expressed in *A. oryzae*, glucose in the culture media of *Saccharomyces cerevisiae* strongly represses the transcription levels of all eight TCA cycle genes. Under AN conditions, the *pckA* gene was specifically expressed as well as those observed in *S. cerevisiae* and in the filamentous fungus *T. reesei*.

3. Biomass-degrading enzymes

Nontoxigenic *Aspergillus oryzae* and *Aspergillus sojae* are industrially cultured on cereals for enzyme production. For these reasons, it is important to elucidate the gene expression profiles of *Aspergilli* fungi on solid-phase surfaces. In the food industry, three biomass materials, such as wheat bran, rice bran, and soybean-curd refuse, are generated in great quantities as waste from wheat, rice, and soybeans. These three waste materials have been used for further enzyme production and have been considered as sources for ethanol production after their enzymatic or chemical degradation. To address enzyme production for biomass use, we used our cDNA microarrays to compare the gene expression profiles of *A. oryzae* cells grown on these three materials. To optimize fermentation, industrial applications ideally require simultaneous monitoring of the expression levels of target genes and those for cellular energy metabolism; therefore, we measured the transcripts of genes in the glycolytic pathway and TCA cycle in addition to those of hydrolytic enzymes (Fig. 2A).

Various genes encoding dietary fiber-degrading enzymes were transcribed in *A. oryzae* cells grown in the wheat-bran

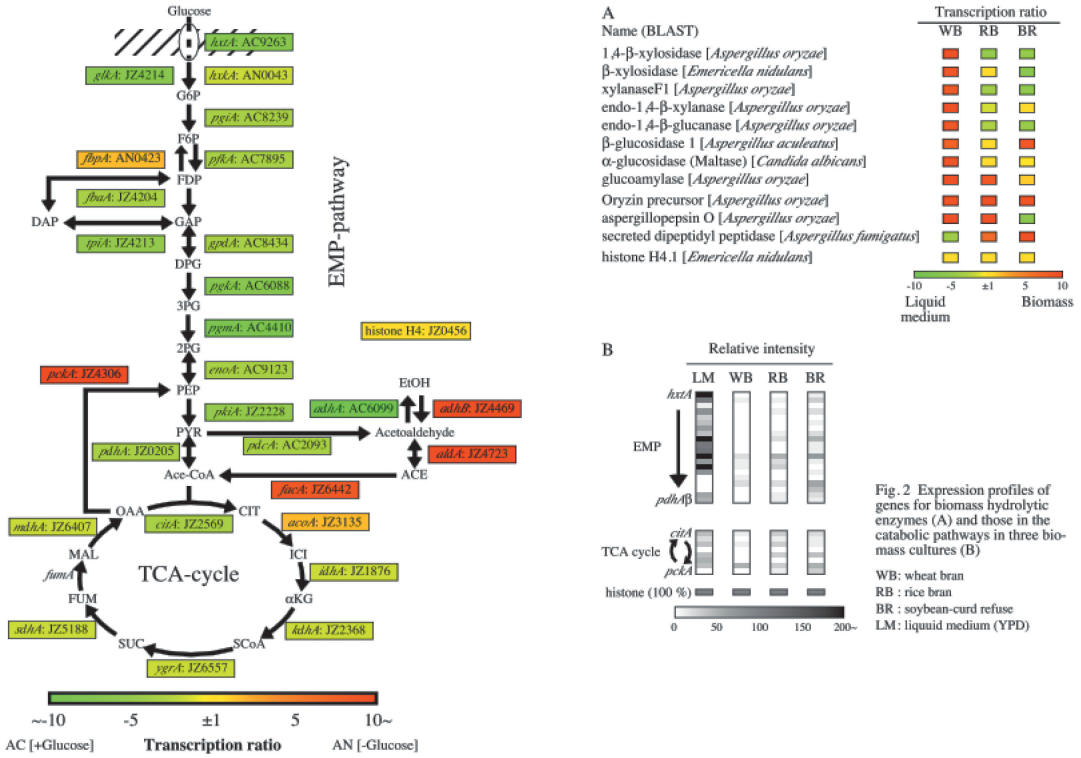

Fig. 2 Expression profiles of genes for biomass hydrolytic enzymes (A) and those in the catabolic pathways in three biomass cultures (B)

Fig. 1. Expression profiles of genes encoding enzymes for the glycolytic pathway (EMP), and tricarboxylic acid (TCA) cycle in *A. oryzae* grown in glucose-rich (AC) and glucose-depleted (AN) media.

solid-phase culture. Soybean-curd refuse generated relatively higher expression levels of proteolytic genes. Proteolytic genes such as oryzin and aspergillopepsin O were well transcribed in the wheat-bran and rice-bran cultures, and the soybean-curd refuse culture showed induced expression of genes for oryzin and dipeptidyl peptidase. In light of these results, the wheat-bran solid-phase medium is better than the other two biomass media for industrial enzyme production. Therefore, our findings validated the use of wheat bran as the medium for Aspergilli fungi by most of the enzyme industry using solid-phase cultures.Further, in the cells grown on the wheat-bran solid-phase culture, the transcription levels of most genes in the EMP and TCA cycle were rather repressed compared with those in the cells grown on the two other biomass media (Fig. 2B). This decreased gene expression might alleviate catabolite repression, which is known to downregulate the transcription levels of many of the hydrolytic enzymes we described.