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Taka-Amylase A in the Conidia of *Aspergillus oryzae* RIB40

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A study of Taka-amylase A of conidia from *Aspergillus oryzae* RIB40 was done. During the research, proteins from conidia and germinated conidia were analyzed using SDS–PAGE, 2-D gel electrophoresis, Western blot analysis, MALDI-TOF Mass spectrometry, and native-PAGE combined with activity staining of TAA. The results showed that TAA exists not only in germinated conidia but also in conidia. Some bands representing degraded products of TAA were detected. Conidia, which formed on starch (SCYA), glucose (DCYA), and glycerol (GCYA) plates, contained mature TAA. Only one active band of TAA was detected after native-PAGE activity staining. In addition, TAA activity was detected in cell extracts of conidia using 0.5 M acetate buffer, pH 5.2, as extraction buffer, but was not detected in whole conidia or cell debris. The results indicate that TAA exists in conidia in active form even when starch, glucose, or glycerol is used as carbon source. TAA might belong to a set of basal proteins inside conidia, which helps in imbibition and germination of conidia.

Key words: *Aspergillus oryzae*; conidia; germinated conidia; soluble proteins; Taka-amylase A

Aspergillus oryzae is a very useful filamentous fungus for Japanese fermented foods such as sake (rice wine), miso (soybean paste), and shoyu (soy sauce). The strain is widely used for the production of various enzymes, homologous and heterologous proteins, in bio-technological industries since it has a great ability to secrete diverse proteins. In the life cycle of this species, there are many stages of development, spanning from germination of conidia to formation of mature conidiophore-bearing chains of conidia. Especially, the late mycelial growth stage is very important for industrial purposes. During this stage many of hydrolytic enzymes are produced, such as glucoamylase,[1] Taka-amylase A,[2,3] proteases,[4] β-galactosidase,[5] lipases,[6] and cellulases[7] which benefits industry with enormous amounts of industrial enzymes for fermented food products. Despite the industrial importance of germination, relatively little is known about them. The nutrition of the conidial germination stage depends on extracellular or/and intracellular hydrolytic enzymes, which help the germ tubes to penetrate into the substrate.

In a previous paper,[8] we reported extracellular enzymes from the early stage of germination by the proteomic approach. Taka-amylase A (TAA), glucoamylase (GLAA), and aspergillopepsin A (PEPA) were the main products during germination. TAA is a useful secreted enzyme that can degrade starch into glucose to fulfill nutrient demands and help in the development of *A. oryzae*. TAA was detected in glucose liquid medium at the fourth hour of germination. On the other hand, EST of *taa* was not detected in the conidia. It is unclear why the conidia of the early germinated stage secreted TAA in the medium. There is no information at the molecular level regarding this. In this study, TAA from conidia of *A. oryzae* RIB40 was analyzed using Western blot analysis of one dimension (1-D), 2-dimension gel electrophoresis (2-DE), the peptide mass fingerprinting method with Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS), and native-PAGE methods. The results show that conidia contained active TAA even when starch, glucose, or glycerol was used as carbon source.

Materials and Methods

Strain and preparation of purified conidia and germinated conidia. *A. oryzae* RIB40 (ATCC 42149) was maintained on potato dextrose agar (PDA) (Nissui, Tokyo, Japan). Conidiating culture was made as described in the previous paper.[8] The conidiating culture medium contained 1% casamino acid, 1% yeast nitrogen base w/o amino acid, 1.5% agar, and 2% sugar. SCYA contained starch, DCYA contained glucose, and GCYA contained glycerol as carbon source. Conidia were inoculated on plates and incubated at 30 °C for 4 d as preculture. Then the conidia were harvested and transferred to new PDA, SCYA, DCYA, and GCYA plates covered with autoclaved filter paper (No. 5C, 90 mm, Advantec). The plates were incubated again at 30 °C for 7 d. The mature conidia were harvested separately and filtered through a P11G25O glass filter, and centrifuged

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