Invasive growth of *Aspergillus oryzae* in rice *koji* and increase of nuclear number

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Abstract

'Rice *koji*' is a solid culture of *Aspergillus oryzae* on steamed rice grains. Multiple parallel fermentation, wherein saccharification of rice by *A. oryzae* and alcohol fermentation by the budding yeast occur simultaneously, lead to the formation of a variety of ingredients of Japanese sake. In sake brewing, the degree of mycelial invasive growth into the steamed rice, called '*haze-komi*', highly correlates with the digestibility and quality of rice *koji*, since the hyphae growing into the rice secrete amylases and digest starch of rice. In this study, we investigated mycelial distribution of GFP-tagged *A. oryzae* in rice *koji* made with different types of rice, such as sake rice and eating rice, with 50 or 90% polishing rate to remove abundant proteins and lipids near the surface. In addition, we compared transcriptomes of *A. oryzae* in the different types of rice *koji*. Finally, we found that *A. oryzae* increases the nuclear number and hyphal width in the course of 1-3 days cultivation. That characteristic could be correlated with the secretory capacity of several enzymes, and be an important selectively acquired characteristic during the breeding in long history.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Figures

Figure 1

Imaging analysis of *A. oryzae* penetration into steamed rice. (A) Koji of different rice races and polishing rates. *Chiyo-nishiki* (eating rice) and *Yamada-nishiki* (sake rice) polished 90% or 50%. Scale bar: 1 cm. (B) Images of section of koji by zoom microscopy. (C) Fluorescent images of *A. oryzae* (H2B-GFP) penetration into the steamed rice. The sections were sliced by cryo-microtome. Scale bars: 200 μm. (D) Distance of fungal penetration from surface in

C90, Y90 and Y50. Error bar: S.D., $n = 20$. *** $P < 0.001$.

A Cryo-microtome

B Confocal-3D

C transparent

Figure 2

Fluorescent imaging analysis of *A. oryzae* penetration inter- and intra-rice cells. (A) Fluorescent images of *A. oryzae* (H2B-GFP) penetration inter- and intra-steamed rice Y50.

The rice cells were shown by UV and BF (bright field). The sections were sliced by cryo-microtome. Hyphal growth inside of rice cells was indicated by arrows. Scale bars: 50 μ m. (B) Confocal-3D imaging of the koji section from Y50 and Y90. *A. oryzae* (green), rice cells (red). Movie 1, 2. Scale bars: 50 μ m. (C) The koji were treated with transparent reagent and imaged by fluorescent microscopy. *A. oryzae* (green), rice cells (purple). Scale bar: 100 μ m.

Figure 3

Fluorescent and SEM imaging analyses of *A. oryzae* penetration intra-rice cells. (A) Fluorescent images of *A. oryzae* (H2B-GFP) penetration intra-steamed rice Y50. Hyphal growth through surrounding space like a furrow in the rice (arrow and dotted line). Scale bars: 20 μ m. (B) SEM images of the koji section. Scale bars: 100 μ m. Cross sections of hyphae from or to holes (yellow arrows). Vertical sections hyphae in ditch (white arrows).

Figure 4

Time-lapse imaging analysis of *A. oryzae* penetration into steamed rice. The sections of koji Y50 (A) and Y90 (B), were imaged every 10 or 20 minutes for 14 hours by fluorescent microscopy. Movie 3, 4. The images at three time points were shown (upper), the images at 14 hours were shown (lower). (A) *A. oryzae* (green), rice cells (red). Some hyphae changed the growth direction after hitting the rice cell shape (arrows). (B) *A. oryzae* (green), rice cells (bright field). Scale bars: 200 μm.

Figure 5

X-ray CT analysis of *A. oryzae* penetration into steamed rice. (A) Image sequence of X-ray CT analysis in C90, Y90 and Y50, Movie 5-7. The rice and fungus are shown in white and yellow, respectively. (B) Fungal volume was calculated from the 3D data. Error bar: S.D., $n = 3$. * $P < 0.05$. The fungal signals were determined by CT value (X-ray absorption).

Figure 6

Increase of nuclear number in *A. oryzae* but not in *A. nidulans*. (A) Images of nuclei in the tip compartment of *A. oryzae*. Scale bars: 50 μm . (B) Confocal-3D imaging of nuclei in the *A. oryzae* tip compartment. Movie 8. Scale bar: 40 μm . (C) Ratio of nuclear distribution pattern classified as class I, II, III (A) in *A. oryzae* (left) and *A. nidulans* (right) at 24-, 48- and 72-hours growth (n = 20). (D) Ratio of hyphal width in *A. oryzae* (left) and *A. nidulans* (right) at 24-, 48- and 72-hours growth (n = 100).

A *A. nidulans*

B *A. oryzae*

Figure 7

Nuclear distribution and mitosis in *A. nidulans*, *A. oryzae* and *N. crassa*. (A) Image sequence of synchronized mitosis in *A. nidulans* observed every minute for 3 hours by fluorescent microscopy. Movie 9. (B, C) Image sequence of nuclei in the *A. oryzae* tip compartment from 1 day incubation (Movie 10) and 3 days incubation (Movie 11). Kymographs of nuclear distribution along the hypha. (D) Image sequence of nuclei in *N. crassa* from Movie 12, 13. The elapsed time is given in minutes (A-D). (A-C) Scale bars: 20 μm , (D) Scale bars: 50 μm .

This is a list of supplementary files associated with this preprint. Click to download.

Movie 3, Y50 t.avi
Movie 11, Ao mitosis2.avi
Movie 12, Nc t1.avi
Movie 13, Nc t2.avi
Movie 10, Ao mitosis.avi
Movie 5, C90 CT.mov
Movie 7, Y50 CT.mov
Movie 6, Y90 CT.mov
Movie 8, Ao nuc 3D.avi
Movie 9, An t.avi
Supplement table.xlsx
Movie 4, Y90 t.avi
Movie 2, Y90 3D.mov
Suppelemental fig.pdf
Movie 1, Y50 3D.mov