Comparative Analysis of *Aspergillus oryzae* with Normal and Abnormal Color Conidia

Mao Ye · Ying Lin · Wenbiao Huang ·
Jinhua Wei

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Abstract This study focuses on the characteristic of strains with anomalous color conidium and compares with normal color conidium. Comparative analysis of enzymes activity and extracellular proteins revealed that *A. oryzae* with anomalous color conidium was not different from the strain with normal color conidium. In addition, *A. oryzae* with anomalous color conidium could not influence the palatability and quality of the soy sauce. These findings provide an insight into *A. oryzae* with anomalous color conidium.

Keywords *Aspergillus oryzae* · Conidium · Color · Characteristic · Soy sauce

Introduction

The filamentous fungus *Aspergillus oryzae* has long been used in the manufacture of traditional fermented foods, such as soy sauce and soybean paste, in East Asia [1–3]. Generally, the conidium of *A. oryzae* show yellow–green color [4], but it sometimes appears in other anomalous color (such as white, or brown) in proliferation process. It has been reported that some genes are involved in formation of conidial pigments in *Aspergillus* species. For example, white and yellow color conidia are caused by the mutations in *wA* and *yA* genes, respectively [5, 6].

However, there exists no report on whether the conidial color affects other characteristics of the fungus. In this paper, we report characteristics of *A. oryzae* with anomalous color conidium, compared with a standard *A. oryzae*, which has normal yellow–green color conidium.

Firstly, *A. oryzae* AS 3.951, which has normal yellow–green color conidium, was obtained from China Center of Industrial Culture Collection (CICC). *A. oryzae* with anomalous color conidium was collected from *A. oryzae* AS 3.951 culture, suspended and serially diluted, and streaked on additional new plates until homogenous colonies were observed (Fig. 1), due to the spontaneous mutation [7, 8].

In the fermentation of traditional foods, *A. oryzae* produces a large number of extracellular enzymes, which greatly affect color, flavor, and aroma of fermented food [9]. The activities of the enzymes: acid protease, neutral protease, alkaline protease, aminopeptidase, carboxypeptidase, glutaminase, α -amylase and pectinase from *A. oryzae* were examined according to the previous methods [10–14]. To our surprise, the enzyme activity of *A. oryzae* with anomalous color conidium was not different from the strain with normal color conidium. A detailed comparison is given in Table 1.

Proteomic analysis had been the most powerful tool for the identification of proteins and suitable for studying the global analysis of extracellular proteomes in an organism [15]. Profiles of the extracellular proteins of *A. oryzae* with anomalous color conidium were obtained by SDS-PAGE electrophoresis [3, 7], and the main protein bands were excised and identified by MALDI TOF/MS analysis. As shown in Fig. 2, the proteins bands were close with different color conidium. Especially, some major extracellular proteins bands, such as α -amylase (TAA), alkaline protease (Oryzin) and hypothetical protein AN3548.2,

M. Ye (✉) · Y. Lin
School of Bioscience and Bioengineering, South China
University of Technology, Guangzhou 51006, China
e-mail: yemao1981@gmail.com

M. Ye · W. Huang · J. Wei
Foshan Haitian Flavouring and Food Co., Ltd.,
Foshan 528000, China

Fig. 1 Isolation of *A. oryzae* with anomalous color conidium. A yellow-green (normal), B brown, C white, D yellow. (Color figure online)

Fig. 2 SDS-PAGE analysis of extracellular proteins from *A. oryzae* with different color conidium in solid-state culture. Line M molecular mass markers, Line 1 yellow-green (normal), Line 2 brown, Line 3 white, Line 4 yellow. The main bands excised and identified by MALDI TOF/MS analysis are labeled. (Color figure online)

were the same in the molecular weight and total amounts of protein. These results not only correspond to the analysis of enzyme activity, but also suggest that features of posttranscriptional secretion regulation of extracellular proteins from *A. oryzae* could not depend on the color of conidium.

The effect of *A. oryzae* with anomalous color conidium on soy sauce production was performed with Tanaka et al. [16]. To evaluate soy sauce production taste, 20 panel members were chosen, and umami and preference were evaluated by paired difference test and by paired preference test, respectively [11]. None out of twenty panel members recognized a difference between the soy sauce made with different color conidium. Therefore, we

can determine that the soy sauce made with different color conidium had no different taste from each other. These results are generally consistent with those observed (Table 1; Fig. 2). The soy sauce production was decided, when *A. oryzae* was used as the starter [17]. Since there was no difference in the kinds of soy sauce production, it could be concluded that the color of conidium could not influence the palatability and quality of the soy sauce.

In this study, the enzymes activity, extracellular proteins and effect on soy sauce show that these strains with different color are not different from each other. Our findings will make a great contribution to understanding of *A. oryzae* with anomalous color conidia.

Table 1 Analysis of enzymes activity from *A. oryzae* with different color conidium in solid-state culture

Strain (U/g)	Yellow-green (normal)	Brown	White	Yellow
Acid protease	21.2 ± 1.2*	20.7 ± 1.1	20.9 ± 1.0	21.4 ± 1.4
Neutral protease	78.2 ± 3.2	77.5 ± 4.5	79.0 ± 3.8	78.3 ± 4.1
Alkaline protease	858 ± 22	842 ± 26	861 ± 30	850 ± 26
Aminopeptidase	2.32 ± 0.33	2.30 ± 0.28	2.35 ± 0.25	2.27 ± 0.36
Carboxypeptidase	2.86 ± 0.13	2.80 ± 0.16	2.82 ± 0.21	2.88 ± 0.15
Glutaminase	3.12 ± 0.10	3.05 ± 0.08	3.16 ± 0.13	3.20 ± 0.20
α-Amylase	255 ± 10	260 ± 13	256 ± 15	252 ± 11
Pectinase	96.4 ± 2.3	95.8 ± 3.5	90.6 ± 8.2	98.1 ± 2.8

* Standard deviation

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