Degradation of formaldehyde by *Aspergillus oryzae*

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Abstract

We examined the capacity of *Aspergillus oryzae*, which is used in the production of traditional fermented foods, to degrade formaldehyde under various conditions. Degradation of formaldehyde was performed using *A. oryzae* mycelia, which were produced from spore suspensions containing 1.0×10^6 spores mL^{-1} . *A. oryzae* actively degraded formaldehyde when cultivated in Czapek-Dox (CD) medium containing 50 and 100 mg l^{-1} formaldehyde, however, formaldehyde degradation was not sustained after an initial decrease in CD medium containing 200 and 300 mg l^{-1} formaldehyde. In CD medium containing 100 mg l^{-1} formaldehyde, *A. oryzae* degraded 89% and 100% of formaldehyde within 24 and 48 h, respectively. In addition, *A. oryzae* was able to repeatedly degrade formaldehyde added to culture medium at 100 mg l^{-1} . Taken together, these findings suggest that *A. oryzae* mycelia have a high capacity for the degradation of formaldehyde in liquid samples.

Keywords: *Aspergillus oryzae*, formaldehyde, bioremediation

INTRODUCTION

Formaldehyde is a highly reactive compound that is widely used in the production of plastics, coatings, and textiles. Thus, large amounts of formaldehyde are often present in industrial wastes that are discharged into the environment¹⁻³⁾. However, formaldehyde is harmful to humans, acting as an irritant of mucosae, skin and respiratory organs³⁾. In rats and mice, exposure to high concentrations of long-term inhaled formaldehyde markedly increases rates of cancer⁴⁾. Formaldehyde causes leukaemia and cancer of the nasopharynx in rodent models, and there is sufficient evidence that formaldehyde is carcinogenic in humans. For this reason, the International Agency for Research on Cancer (IARC) has classified formaldehyde as a carcinogenic substance to humans (Group 1)³⁾.

According to Pollutant Release and Transfer Register (PRTR) data, 8000 t of

formaldehyde was released into the environment in 2010, and industries that produce or utilize formaldehyde annually generate 630 t of waste containing this compound⁵⁾. The safe level of formaldehyde, as reported by the World Health Organization, is below 0.05 mg l^{-1} , and the Japanese Ministry of Health, Labour and Welfare has set the drinking water quality standard for formaldehyde levels as 0.08 mg l^{-1} ⁶⁻⁷⁾. Considering the high cytotoxicity of formaldehyde to humans and other organisms in the environment, effective methods for the removal of formaldehyde from water are needed.

Formaldehyde can be removed from wastewater by both physicochemical and biological treatment processes⁸⁻¹⁰⁾. In physicochemical approaches, activated carbon is commonly used as an adsorbent for formaldehyde, however, upon saturation of the surface, formaldehyde is not effectively removed⁹⁾. In contrast, biological treatment processes typically

involve microbes that function as efficient and renewable catalysts for environmental purification¹¹⁾. To date, a number of formaldehyde-degrading microorganisms have been identified and include members of the genera *Debaryomyces*, *Trichosporon*¹²⁾, *Escherichia*¹³⁾, *Brevibacillus*¹⁴⁾, *Rhodococcus*¹⁵⁾, *Corynebacterium*¹⁶⁾, *Mycobacterium*¹⁷⁾, *Acetobacter*, *Pseudomonas*¹⁸⁾, ¹⁹⁾ *Penicillium*¹⁰⁾, *Aspergillus*¹⁰⁾, *Trichoderma*²⁰⁾, and *Paecilomyces*²¹⁾. As characteristics of the microorganisms reported to date, bacteria are generally able to degrade high concentration of formaldehyde in a short period of time²²⁾. On the other hand, fungi are generally able to degrade high concentration of formaldehyde in a relatively long period of time, however fungi have stronger environmental adaptability compared to bacteria²⁰⁾. We have also demonstrated that the fungus *Aspergillus oryzae* is able to degrade formaldehyde²³⁾. Although the formaldehyde degradation ability of *A. oryzae* is inferior to that of other microorganisms reported to date, *A. oryzae* is advantageous for use in environmental biodegradation processes because its safety has been established over the past several hundred years in the production of traditional fermented Japanese foods, such as soy sauce, miso and sake, and has been confirmed as a safe by the USA Food and Drug Administration. Moreover, the *A. oryzae* genome was completely sequenced in 2005²⁴⁾ and is expected to be a reservoir for enzymes with desirable biocatalytic activities. Thus, we were interested in examining formaldehyde degradation in more detail in *A. oryzae*.

In the present study, we investigated the capacity and conditions for *A. oryzae* to degrade formaldehyde.

MATERIALS AND METHODS

Microorganism and culture conditions

Aspergillus oryzae RIB40 was used in this study. *A. oryzae* RIB40 (National Research Institute Culture Stock, ATCC42149) was selected because the complete genome sequence has been determined and this strain shares typical characteristics in terms of morphology, growth, and amylase production with industrial strains used for sake brewing, and also has the potential to

produce strong protease activity, which is important in soy sauce fermentation²⁵⁾. To produce mycelia for the degradation of formaldehyde, spore suspensions containing 1.0×10^6 spores ml^{-1} were prepared. Pre-cultures were performed in 100 ml -Erlenmeyer flasks containing 20 ml YPD medium (10 g yeast extract, 20 g polypeptone, and 20 g dextrose in 1 l sterile deionized water) inoculated with 1 ml spore suspension, and were incubated aerobically at 30 °C on a rotary shaker at 100 rpm for 24 h. The mycelia produced in the preculture were washed with sterile deionized water, and were then transferred into 40 ml Czapek-Dox (CD) medium, YPD medium, and sterile deionized water supplemented with and without 100 mg l^{-1} formaldehyde, and were further incubated at 30 °C with shaking at 100 rpm. CD medium contained 20 g sucrose, 3 g NaNO_3 , 1 g K_2HPO_4 , 0.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g KCl, and 0.01 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in 1 l sterile deionized water.

Estimation of formaldehyde concentration

Formaldehyde concentration was estimated using the Hantzsch reaction between acetylacetone solution (15% ammonium acetate, 0.3% acetic acid, and 0.2% acetylacetone) and formaldehyde, which forms 3,5-diacetyl-1,4-dihydrolutidine (DDL, $\lambda_{\text{max}} = 415 \text{ nm}$), as reported by Nash²⁶⁾. For the measurements, samples containing formaldehyde (1 ml) were added to acetylacetone solution (1 ml) and reacted at 60 °C for 10 min. The absorbance of DDL was then measured at 415 nm using a UV-Vis/NIR (Hitachi).

Effect of formaldehyde concentration on degradation by *A. oryzae* To determine the concentration of formaldehyde degraded efficiently or do not affect formaldehyde degradation rates by *A. oryzae*, mycelia were cultured in CD medium containing 50, 100, 200, 300, 400, and 500 mg l^{-1} formaldehyde. The cultures were incubated at 30 °C on a rotary shaker at 100 rpm for 24 h, and formaldehyde concentrations of the culture medium were then determined.

Sustainability of formaldehyde degradation by *A. oryzae* The time course for formaldehyde degradation by *A. oryzae* was examined. In this experiment, *A. oryzae* was

cultured for 24 h in CD medium containing 100 mg l^{-1} formaldehyde. The transfer of mycelia to fresh CD medium containing 100 mg l^{-1} formaldehyde was repeated several times. The concentration of formaldehyde remaining in the medium at the time of each transfer was measured.

RESULTS AND DISCUSSION

Effect of culture medium nutrient composition on formaldehyde degradation ability of *A. oryzae* *A. oryzae* degraded formaldehyde in both types of nutrient media (YPD and CD) and in sterile deionized water, however, formaldehyde was degraded more efficiently in the nutrient media compared to sterile deionized water (Fig. 1). This result indicated that *A. oryzae* produced a higher abundance of degradative enzymes when cultured in nutrient media (YPD and CD). The culture medium nutrient composition also appeared to affect the degradation of formaldehyde by *A. oryzae*. In contrast to the uninoculated control sample of CD medium,

Fig. 1 Degradation of formaldehyde by *A. oryzae* in YPD medium, Czapek-Dox medium and sterile deionized water. Mycelia were cultured in sterile deionized water, and YPD and Czapek-Dox media containing 100 mg l^{-1} formaldehyde at 30 °C with shaking at 100 rpm. For YPD medium, the amount of formaldehyde adsorbed to components in the medium was subtracted from the reported values. Error bars represent the standard error of the mean calculated for three replicates.

Symbols: ●: *A. oryzae* in sterile deionized water ■: *A. oryzae* in CD medium ▲: *A. oryzae* in YPD medium

in which the formaldehyde concentration remained unchanged, the concentration of approximately 30 mg l^{-1} formaldehyde decreased in the uninoculated control sample of YPD medium (data not shown). This result was attributable to components in the YPD medium, such as peptone and yeast extract, which bind to formaldehyde via amino groups^{27)–28)}. For this reason, the amount of formaldehyde adsorbed by components in the YPD medium of the control sample was subtracted from the values found in the test samples. As a result of this normalization, formaldehyde was found to be degraded more efficiently in CD medium compared to YPD medium.

Effect of concentration on formaldehyde degradation by *A. oryzae* The efficacy of bioremediation is limited under conditions in which the concentration of the pollutant is toxic to the degradative microorganisms. Formaldehyde damages DNA, and cause mutations in the genomes of microorganisms and mammalian cells²⁹⁾. In the present study, *A. oryzae* actively degraded formaldehyde when cultured in CD medium containing 50 and 100 mg l^{-1} formaldehyde. Moreover, at these concentrations, *A. oryzae* completely degraded the formaldehyde in the CD medium within 48 h (Fig. 2). However, degradation

Fig. 2 Effect of concentration on formaldehyde degradation by *A. oryzae*. Mycelia were cultured in CD medium containing 50, 100, 200, and 300 mg l^{-1} formaldehyde at 30 °C at 100 rpm. Error bars represent the standard error of the mean calculated for three replicates.

Symbols: ◆: 300 mg l^{-1} ■: 200 mg l^{-1} ▲: 100 mg l^{-1} ●: 50 mg l^{-1}

was not sustained past an initial decrease in medium containing high formaldehyde concentrations at $\geq 200 \text{ mg l}^{-1}$. These results indicate that formaldehyde concentrations at $\leq 100 \text{ mg l}^{-1}$ in CD medium do not affect formaldehyde degradation rates by *A. oryzae*. Therefore, in subsequent experiments, formaldehyde degradation was analyzed using CD medium supplemented with 100 mg l^{-1} formaldehyde.

Sustainability of formaldehyde degradation by *A. oryzae* *A. oryzae* would remain viable and completely degrade 100 mg l^{-1} formaldehyde when repeatedly transferred to the fresh culture medium. The sustainability of formaldehyde degradation by *A. oryzae* in CD medium is shown in Fig. 3. *A. oryzae* was able to repeatedly degrade formaldehyde in cultures containing viable cells. Furthermore, *A. oryzae* degraded formaldehyde more efficiently after each transfer of mycelia to fresh CD medium containing 100 mg l^{-1} formaldehyde, and formaldehyde was completely degraded within 24 h after the third transfer of mycelia to the fresh culture medium containing 100 mg l^{-1} formaldehyde. The observed degradative patterns are attributed to the continued growth of *A. oryzae* and intracellular accumulation of formaldehyde-degrading enzymes.

It is generally known that formaldehyde is a metabolic intermediate in methanol

metabolism. In this pathway, formaldehyde is oxidized to formate, which is then oxidized to carbon dioxide. As most animals, plants, and microorganisms use this pathway³⁰⁾, it is likely that *A. oryzae* consumes formaldehyde via enzymes of the methanol metabolic pathway. To determine the *A. oryzae* enzyme(s) involved in the degradation of formaldehyde, gene-knockout experiments will be conducted in future studies.

CONCLUSION

We examined the potential of *A. oryzae* to remove formaldehyde from water and nutrient media (YPD and CD). We confirmed that *A. oryzae* efficiently degrades formaldehyde in nutrient medium. In CD medium containing 50 and 100 mg l^{-1} formaldehyde, *A. oryzae* was able to efficiently degrade formaldehyde. Moreover, at 100 mg l^{-1} formaldehyde, *A. oryzae* was able to completely degrade formaldehyde over five transfer/degradation cycles. Taken together, these findings suggest that *A. oryzae* pellets have a high capacity for the degradation of formaldehyde in liquid samples.

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Fig. 3 Sustainability of formaldehyde degradation by *A. oryzae*. Formaldehyde degradation in cultures of *A. oryzae* was monitored after repeated supplementation of CD medium at 30°C at 100 rpm . Error bars represent the standard error of the mean calculated for three replicates. Symbols: ●: Control ○: *A. oryzae* in CD medium

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