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Uptake and transformation of arsenic during the vegetative life stage of terrestrial fungi

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ABSTRACT

Many species of terrestrial fungi produce fruiting bodies that contain high proportions of arsenobetaine (AB), an arsenic compound of no known toxicity. It is unknown whether fungi produce or accumulate AB from the surrounding environment. The present study targets the vegetative life stage (mycelium) of fungi, to examine the role of this stage in arsenic transformations and potential formation of AB. The mycelia of three different fungi species were cultured axenically and exposed to AB, arsenate (As(V)) and dimethylarsinoyl acetic acid for 60 days. *Agaricus bisporus* was additionally exposed to hypothesized precursors for AB and the exposure time to As(V) and dimethlyarsinic acid was also extended to 120 days. The mycelia of all fungi species accumulated all arsenic compounds with two species accumulating significantly more AB than other compounds. Few biotransformations were observed in these experiments indicating that it is unlikely that the mycelium of the fungus is responsible for biosynthesizing AB.

1. Introduction

Over 50 arsenic compounds have been identified in the environment and the toxicity of arsenic is greatly dependent on its chemical form (Reimer et al., 2010), but the only arsenic compound that is considered to be non-toxic is arsenobetaine (AB) (Kaise et al., 1985). AB is predominantly found in marine organisms where it comprises a large proportion of the total arsenic. Therefore two of three current proposed pathways (Fig. 1) for the formation of AB originate from studies of the marine environment (Caumette et al., 2012).

Currently, the main hypothesis for AB formation is from the degradation products of dimethylated or trimethylated arsenosugars, although the latter are not as commonly found in the environment. Dimethylated arsenosugars are thought to be precursors because the dietary sources for marine organisms (phytoplankton and marine kelp) contain elevated levels of these compounds (Edmonds et al., 1997). It has been proposed that the formation of AB may occur from the degradation of dimethylated arsenosugars to arsenocholine (AC), which is then converted to AB (Pathway 1 in Fig. 1). The last step in Pathway 1, the conversion of AC to AB, has been demonstrated in laboratory studies within mice, rats and rabbits (Marafante et al., 1984). However shrimp and sheep did not degrade ingested dimethylated arsenosugars to AC (Francesconi et al., 1999; Hansen et al., 2003), and degradation in other circumstances has not yet been reported. Thus the formation pathway from arsenosugars to AB may not involve AC as an intermediate. Dimethylarsinoyl acetic acid (DMAA) has been proposed as a potential intermediate similar to AC (Pathway 2 in Fig. 1) and has been demonstrated to be a major degradation product of arsenosugars in sheep (Hansen et al., 2003). DMAA was also a precursor to AB in laboratory studies involving lysed bacteria extracts (Ritchie et al., 2004). Fish fed DMAA, however, did not form AB (Francesconi et al., 1989). The third proposed formation pathway for AB (Pathway 3 in Fig. 1) offers a more direct route by simple methylated compounds involving dimethylarsinous acid (DMA(III)) and 2-oxo acids, glyoxylate and pyruvate, to form DMAA and then AB (Edmonds, 2000).

In contrast to the marine environment, few terrestrial organisms contain AB and, when present, it comprises lower proportions of total arsenic. The exception to this trend is the fruiting bodies, or mushrooms, of terrestrial fungi in the Basidiomycetes class. Terrestrial species in this class of fungi can grow through asexual reproduction, producing the vegetative life stage (mycelium), and through sexual reproduction, undergoing a reproductive life stage, during which fruiting bodies, or mushrooms, are produced for spore dispersal. The fungus spends most of its life in the mycelium

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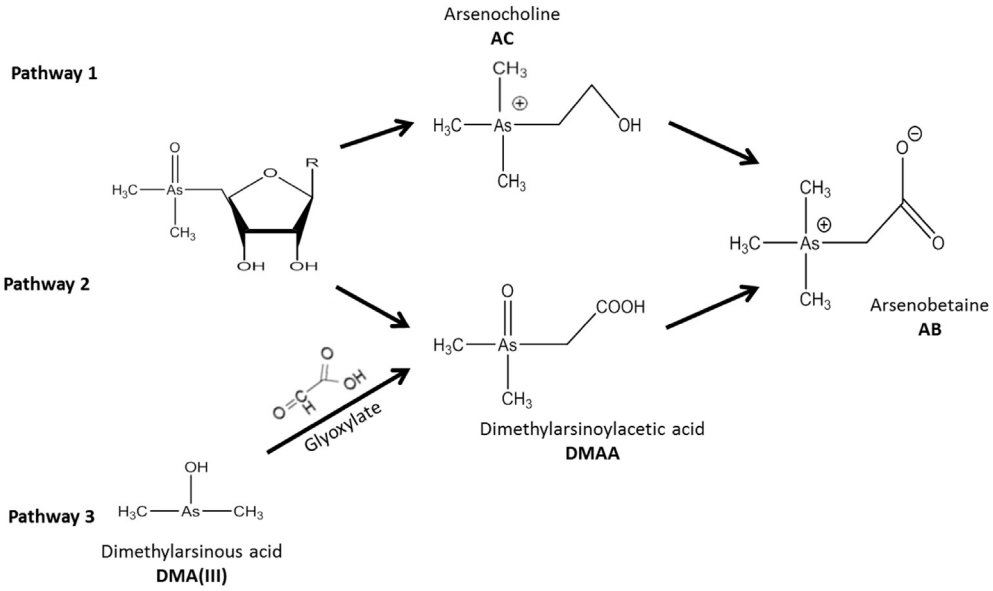

Fig. 1. Current proposed formation pathways for arsenobetaine (AB).

form until environmental conditions specific to each fungus species trigger fruiting body development (Stamets, 2000), which is seasonal (10–14 days) in the wild (Kalač and Svoboda, 2000). Commercially cultivated species such as *Agaricus bisporus* (the button mushroom available widely in grocery stores) are grown under conditions that allow multiple fruiting body production events, or flushes (Kües and Liu, 2000).

Mushrooms are the main part of fungus species that have been collected and analyzed from the environment, attributable to their visibility and ease of picking and identification. They have been found to contain a variety of organoarsenic compounds, and compared with most other terrestrial organisms, a higher proportion of AB (Reimer et al., 2010). This abundance of AB in terrestrial fungi provides a simpler study system for the formation of AB than marine organisms, making them a desirable target organism for this purpose.

The role of AB in mushrooms is still unknown, but localization of AB to the cap and outer stalk has led to the hypothesis that AB may also play an osmolytic role, since the fruiting bodies rely on osmotic regulation for spore dispersal (Reimer et al., 2010). The life stage at which the fungus produces AB is still unknown, as is whether the fungus, alternatively, accumulates AB from the surrounding environment. That is, the surrounding microbial community in the soil may produce AB or its precursors. Very few studies report the detection of arsenosugars in mushrooms (Koch et al., 1999, 2000) and no arsenosugars were found in the growth material when *A. bisporus* was grown on arsenate (As(V)) treated material (Smith et al., 2007), making it unlikely that AB is produced from the degradation of arsenosugars, either through the fungus or the surrounding microbial community. In the aforementioned study (Smith et al., 2007), the fungus-free growth material contained monomethylarsonic acid (MMA), DMA and mainly trimethylarsine oxide (TMAO) likely produced by the microbial community; we hypothesize that these compounds could then be accumulated and further transformed by the fungus.

The present study targets the vegetative, mycelium life stage of terrestrial fungi, to examine the role of this stage in arsenic transformations, especially to AB. The present study targets the mycelium life stage, grown in axenic laboratory culture, to obtain controlled conditions and to exclude the influence of the microbial community. The mycelia of three fungus species from the Basidiomycetes class were cultured with different arsenic compounds: *A. bisporus*, known to contain AB as the major arsenic compound in its fruiting bodies when cultivated; *Sparassis crispa* (common name cauliflower mushroom), known to contain AC in fruiting bodies (Šlejkovec et al., 1997); and *Suillus luteus* (common name Slippery Jack), a species from a genus known to contain no AB but primarily DMA in fruiting bodies (Kuehnelt and Goessler, 2003). All mycelia were exposed to As(V), DMAA and AB. *A. bisporus* was additionally exposed to MMA, DMA and TMAO, based on previous findings on fruiting body laboratory culture of this fungus (Smith et al., 2007). DMAA was selected to test if the fungus could transform DMAA to AB (Pathway 3 in Fig. 1). Ideally the mycelium would also be exposed to arsenosugars to test Pathway 1 and 2 in Fig. 1; however no authentic pure sugar standards were available to us, and algal extracts commonly used to identify arsenosugars in HPLC-ICP-MS analysis would not be suitable because they also contain As(V) and DMA (Madsen et al., 2000). AC was also not used as a treatment because it is infrequently found together with AB (Šlejkovec et al., 1997).

2. Methods

2.1. Fungus cultures

Chemicals and reagents are listed in the supplementary information. Fungus species included *A. bisporus* (ATCC® 10892™), *S. crispa* (ATCC® 34491™) (both grown in potato dextrose broth), and *S. luteus* (ATCC® MYA-4759™) (yeast mold broth). Mycelia were revived and grown on suggested media until they had over grown the petri dishes. Three pieces of approximately 1 cm^2 pieces of agar with mycelium were added to the corresponding broth. Mycelia were shaken at 140 rpm at 20 °C with all manipulations carried out under a biohood to prevent contamination.

The experimental design is summarized in Table 1. All treatments were triplicated and included controls with mycelia grown in arsenic-free media (inoculated negative control); without mycelia in arsenic-containing media (uninoculated positive control); and arsenic- and mycelia-free media (uninoculated negative control). Arsenic compounds were aseptically added to the broth