OP-2

Structural and functional analysis of a novel arsenic compound produced by a bacterium isolated from the rice root

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Abstract

The origin of methylated As in rice grains has been clarified in resent studies. Some soil bacteria which have As methyltransferase gene, ArsM, involve in As methylation in the rice rhizosphere, and the plant just uptakes methylated As.

In our study, we isolated two types of bacteria, the one produces methylated As form iAs and the other a novel organoarsenic compound (named as 'AST'). AST is not detected in rice grain but it is interesting As chemical form which may indicate a complex metabolism in the rhizosphere. Here we characterized the chemical structure and effects of AST to rice plants or other bacteria.

AST purified from the As containing culture of *Burkholderia gladioli* strain GSRB05 was determined as 2-amino-4-(hydroxymethylarsinoyl)butanoic acid by MS and NMR experiments. From the result, it was appeared that AST is an amino acid derivative similar to the structure of glufosinate which is well-known as herbicide.

A little amount of AST was absorbed by rice plants in uptake experiments, but E. coli cells in a minimal medium which lacks amino acids absorbed AST more than As(III) and the growth was worse. AST may function as an anti-bacterial metabolite.

Keywords: Arsenic, metabolism, rhizosphere

Introduction

Arsenic chemical forms accumulated in rice grains are not only inorganic arsenicals (iAs) but also methylated arsenicals, especially dimethylarsinic acid (DMA)（Schoof et al., 199）. Recent studies indicate that DMA is produced by soil bacteria and rice plants just uptake it (Lomax et al., 2012; Jia et al., 2013; Zhao et al., 2013). We also have isolated a bacterium transform iAs to DMA from the rice rhizosphere（Kuramata et al. 2014）. On the other hand, we found another bacterium which metabolized iAs to an unknown organic arsenical (named as 'AST' in the later studis) from the same soil sample.

Although AST is not detected in rice grains, we characterized the chemical structure and effects of AST to rice plants or other bacteria for purpose of elucidation of arcenic dynamics in the rice rhizosphere.

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Results and discussions

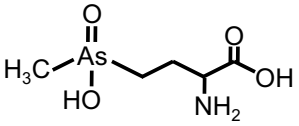

Fig. 1. Planar structure of AST

A bacterial strain GSRB05 producing AST separated from rhizosphere of rice plant was identified as *Burkholderia gladioli* by 16S *rRNA* gene sequencing.

The molecular formula of AST was decided as $C_5H_{12}O_4NAs$ by the ultra-high performance liquid chromatography coupled high resolution mass spectrometry (UPLC/HRMS). Additionally, results of one and two-dimensional nuclear magnetic resonance (NMR) experiments indicated that the structure was 2-amino-4-(hydroxymethylarsinoyl)butanoic acid (Fig. 1). This is a novel arsenic compound, we named Arsinothricin because it is an arsenic mimetic of the herbicide phosphinothricin (PPT).

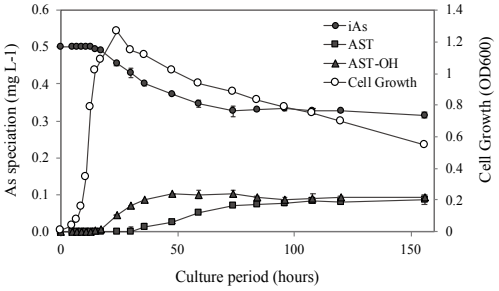

Fig. 2. Time-course of arsenic transformation by *B. gladioli* GSRB05.

AST has a monomethylarsinic partial structure, but it is not synthesized from monoarsonic acid *in B. gladioli* GSRB05. Perhaps it was considered that the AST biosynthesis pathway would be similar to that of PPT（Schwartz et al. 2004）. In the time-course experiment of AST production by GSRB05, it was observed that another unknown arsenical was produced earlier than AST by HPLC/ICP-MS analysis (Fig. 2). We referenced PPT biosynthesis pathway （Schwartz et al., 2004）and hypothesized that the arsenic compound had a structure in which the methyl group bound to the As atom in AST was replaced with a hydroxyl group. From results of multiple-reaction monitoring UPLC/MS/MS, it was judged to be 2-amino-4-(dihydroxyarsonoyl)butanoic acid (AST-OH). Thus, AST biosynthesized would be initiated from iAs and methylation of arsenic atom is occurred at down-stream of the pathway.

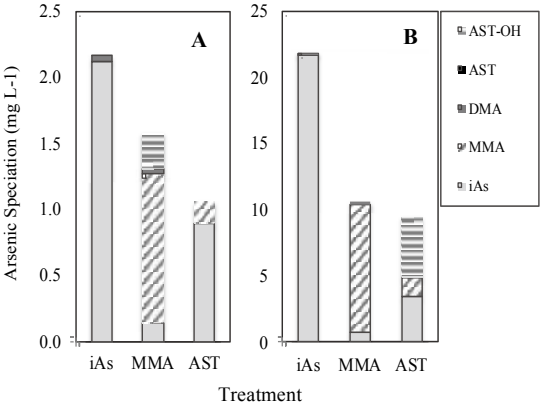

Fig. 3. Arsenic speciation in the shoot (A) and root (B) rice plants treated each arsenicals for 7 days.

Biological activity of AST was considered like as PPT because of their structural similarity. At first, we investigated whether rice plants uptake AST or not, and then AST was not detected in the both of shoot and root (Fig. 3). Next, we treated AST to *Escherichia coli* cells, then AST accumulation was much more than arsenite in M9 minimal medium though a little amount AST was accumulated in the cells cultured in LB medium (Fig. 4). Additionally, E. coli cells did not grow in the AST containing M9 medium (Fig. 5). Although further experiments are necessary, AST may also have a toxicity similar to PPT.

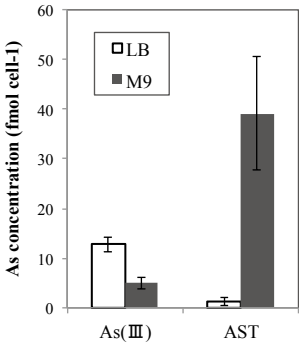

Fig. 4. Arsenic accumulation in *E. coli* cells exposed arsenite or AST in LB medium or M9 minimal medium.

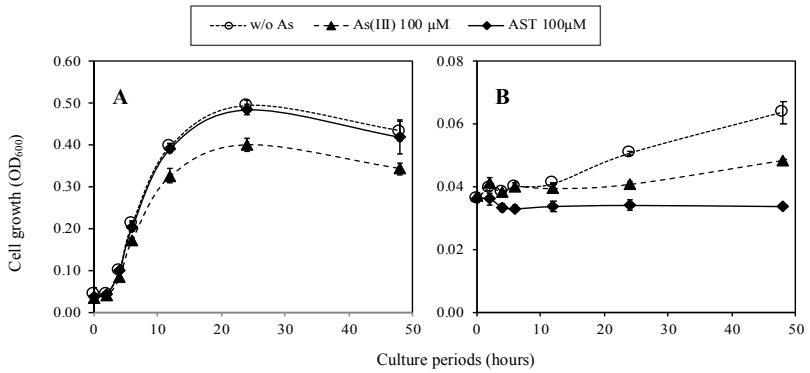

Fig. 5. Growth of *E. coli* cells exposed arsenite or AST in LB medium (A) or M9 minimal medium (B).

Materials and methods

Bacteria were separated from rhizosphere of rice plant grown on a pot (diameter 7.5 cm) filled with a paddy soil that contained 8 mg kg^{-1} of 1 M HCl-extractable arsenic, by the agar plate dilution method (Kuramata et al. 2014). Arsenic species were separated and detected by HPLC/ICP-MS with an ODS-3 column and a mobile phase containing 3 mM malonic acid and 5 mM tetrabutylammonium hydroxide in 5 % (v/v) methanol (pH 5.6).

AST was purified from a *B. gladioli* GSRB05 culture solution with size-exclusion and anion-exchange chromatographies (Kuramata et al. 2016).

AST tolerance and accumulation assay was performed using rice seedlings grown in sterile condition and *Escherichia coli* DH5 α pre-cultured in Luria-Bertani (LB) or M9 minimal liquid medium. Rice seedlings grown in Murashige and Skoog liquid medium for 1 week approximately were treated with 2.67 μM of each arsenicals. On the other hand, *E. coli* cells grown in each medium for over-night were resuspended to fresh medium and treated with 100 μM of each arsenicals.

After arsenic treatment, rice samples separated to shoots and roots and bacterial cells were dried and then digested with 60% nitric acid at 105℃. Digested samples were diluted and measured arsenic concentration using ICP-MS.

References

Kuramata,M., Sakakibara,F., Kataoka,R., Abe,T., Asano,M., Baba,K., Takagi,K., and Ishikawa,S. (2015), Arsenic biotransformation by Streptomyces sp. isolated from rice rhizosphere, Environmental Microbiology, 17, 1897-1909pp.

Lomax,C., Liu,W.J., Wu,L., Xue,K., Xiong,J., Zhou,J., McGrath,S.P., Meharg,A.A., Miller,A.J, Zhao,F.J. (2012). Methylated arsenic species in plants originate from soil microorganisms, New Phytologist, 193, 665-672pp.

Jia,Y., Huang,H., Zhong,M., Wang,F.H., Zhang,L.M., and Zhu,Y.G. (2013). Microbial arsenic methylation in soil and rice rhizosphere, Environmental Science & Technology, 47, 3141-3148pp.

Schoof,R.A., Yost,L.J., Eickhoff,J., Crecelius,E.A., Cragin,D.W., and Meacher,D.M. (1999) A market basket survey of inorganic arsenic in food, Food and Chemical Toxicology, 37, 839-846pp.

Schwartz,D., Berger,S., Heinzelmann,E., Muschko,K., Welzel,K., and Wohlleben,W. (2004). Biosynthetic gene cluster of the herbicide phosphinothricin tripeptide from Streptomyces viridochromogenes Tü494, Applied and Environmental Microbiology, 70, 7093-7102pp.

Zhao,F.J., Harris,E., Yan,J., Ma,J., Wu,L., Liu,W., Steve,P.M., Jizhong,Z., and Yong-Guan,Z. (2013). Arsenic methylation in soils and its relationship with microbial arsM abundance and diversity, and as speciation in rice, Environmental Science & Technology, 47, 7147-7154pp.